

# Kongeriget Danmark

Patent application No.: PA 2002 01634  
Date of filing: 29 October 2002  
Applicant: Borean Pharma A/S  
(Name and address) Gustav Wieds Vej 10  
DK-8000 Århus C  
Denmark

Title: Binders for Trimeric Cytokines

IPC: -

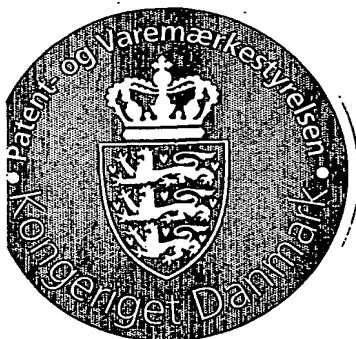
This is to certify that the attached documents are exact copies of the above mentioned patent application as originally filed.

**PRIORITY DOCUMENT**  
SUBMITTED OR TRANSMITTED IN  
COMPLIANCE WITH  
RULE 17.1(a) OR (b)

Patent- og Varemærkestyrelsen  
Økonomi- og Erhvervsministeriet

21 November 2003

  
Bo Zillo Tidemann



**BEST AVAILABLE COPY**

**Binders for Trimeric Cytokines**

PVS

***Field of the invention***

The present invention pertains to the provision of trimeric binders which bind to trimeric cytokines in a manner such that upon binding, all receptor binding sites of the trimeric cytokine are substantially blocked, and hence the potential biological activity of the trimeric cytokine is suppressed. In one aspect the invention relates to trimeric binders capable of binding to cytokines of the Tumour necrosis factor ligand superfamily, including tumour necrosis factor (TNF).

***Background of the invention and prior art***

Cytokines are small secreted polypeptides from higher eukaryotes which are responsible for intercellular signal transduction and which affect the growth, division and functions of other cells. They are potent, pleiotropic polypeptides that, e.g. via corresponding receptors, act as local or systemic intercellular regulatory factors, and therefore play crucial roles in many biologic processes, such as immunity, inflammation, and hematopoiesis (formation and development of blood cells in the bone marrow). Cytokines are produced by diverse cell types including fibroblasts, endothelial cells, macrophages/monocytes, and lymphocytes. To date, a large number of cytokines have been identified, including interferons, tumour necrosis factors, interleukins and lymphokines. They differ from classical hormones in that they are produced by a number of tissues or cell types rather than by specialized glands.

The modulation of cytokine-receptor interactions can be a useful mechanism for therapeutic intervention in various diseases and pathologies. Soluble binding proteins, which can interact with cytokines, can potentially sequester the cytokine away from the receptor, thereby reducing or preventing the activation of that particular receptor pathway.

Certain cytokines occur as multi-subunit complexes containing multiple copies of the same subunit. Macrophage migration inhibitory factor (MIF) and proteins within the tumour necrosis factor ligand super family (TNFLSF), such as Tumour necrosis factor (TNF;

TNFLSF member 2) and lymphotoxin alpha (LT-alpha; TNFLSF member 1), occur as homotrimers formed by three identical subunits. Many of these trimeric cytokines are known to be involved in signal transduction and to act as regulatory factors, and to be involved in many different types of diseases and medical indications.

The trimeric cytokines of the TNF ligand super family are known to control and orchestrate the immune and inflammatory responses at several levels. It is also known that TNF ligand super family members are associated with several disease conditions, such as acute or chronic inflammatory conditions. Presently, the TNF ligand super family has at least 17 recognised ligands, including LTA (lymphotoxin alpha; TNFLSF member 1), TNF (tumor necrosis factor; TNFLSF member 2), LTB (lymphotoxin beta; TNFLSF member 3), OX-40L (TNFLS member 4); CD40L (TNFLS member 5); FasL (TNFLS member 6); CD27L (TNFLS member 7); CD30L (TNFLS member 8); 4-1BB-L (TNFLS member 9); TRAIL (TNFLS member 10); RANKL (TNFLS member 11); TWEAK (TNFLS member 12); APRIL (TNFLS member 13); BAFF (TNFLS member 13B); LIGHT (TNFLS member 14); VEGI (TNFLS member 15); and GITRL (TNFLS member 18).

Evidence exist that the signalling unit of the trimeric cytokines of the TNF ligand super family and their corresponding receptors, is in the form of three receptors and three ligands assembled as a hexameric complex in which a single cytokine trimer binds to three receptor molecules (Bodmer et al. 2002, TRENDS in Biochemical Sciences 27(1), pp. 19-26). Before becoming part of this hexameric complex, the receptors exist in monomeric form. This general mechanism is illustrated in Figure 1.

TNF (TNFLSF member 2) is one of the principal mediators of the immune and inflammatory response, and it is e.g. known to have an important role in the pathogenesis of rheumatoid arthritis, which is a common autoimmune inflammatory disease that affects approximately 0.5-1% of the human population. Additionally, TNF is also known to be involved in the pathogenesis of a wide range of disease states, including endotoxin shock, cerebral malaria and graft-versus-host reaction. TNF is produced by a number of cell types, mainly by activated macrophages. The soluble form of TNF consists of three

identical 17 kD protein subunits, whereas the membrane bound form consist of three identical 26 kD subunits. TNF is also previously known as "TNF-alpha" and "cachectin".

TWEAK (TNFLSF member 12) was recently found to be a direct and strong inducer of angiogenesis (formation and growth of blood vessels) as it was observed that picomolar concentrations of TWEAK promote proliferation of normal endothelial cells and that TWEAK induces angiogenesis in an *in vivo* rat cornea model (Lynch et al., 1999, The Journal of Biological Chemistry, 274(13) pp. 8455-8459). In particular angiogenesis is essential for the growth and persistence of solid tumours and their metastases, and it is also known to be involved in other pathological conditions such as diabetic retinopathy, psoriasis, contact dermatitis and restenosis.

In US20020037852A1 the trimeric cytokine BAFF (TNFLS member 13B) was described to be expressed by T cells and dendritic cells for the purpose of B-cell co-stimulation and consequently may play an important role in the control of B cell function. It is suggested therein that BAFF and its receptor may have anti-cancer and immunoregulatory applications as well as uses for the treatment of immunosuppressive disorders such as HIV.

APRIL (TNFLS member 13) is a trimeric cytokine that binds to the receptor TNFRSF13B and to TNFRSF17. It has recently been shown that the addition of recombinant APRIL to various tumor cells stimulates their proliferation and hence APRIL may be implicated in the regulation of tumor cell growth (Hahne M., et al, 1998; J. Exp. Med. 188:1185-1190). It has also been suggested that APRIL may be involved in monocyte/macrophage-mediated immunological processes.

Furthermore, cytokines within the TNF super family have also been described to be involved in hereditary diseases such as hyper IgM syndrome, type I autoimmune lymphoproliferative syndrome, TNF-R1-associated periodic fever syndrome, hypohidrotic ectodermal dysplasia and familial expansile osteolysis.

Several attempts have been made in order to find and develop suitable antagonists and binders for trimeric cytokines which are capable of binding to specific trimeric cytokines and thereby preventing the trimeric cytokines from binding to e.g. cell membrane bound receptors and hence suppressing the activation of that particular receptor pathway.

One example is antagonists against the trimeric cytokine TNF (TNFLSF member 2). Presently, two types of TNF antagonists are commercially available, namely Infliximab (Remicade) and Eternarcept (Enbrel) which have both received marketing authorization in the United States and Europe for treatment of rheumatoid arthritis. The two products have also been shown to be effective for the treatment of psoriasis and Crohn's disease. Infliximab is a chimeric antibody with murine variable regions and human IgG1 and  $\kappa$  constant regions, which neutralizes the biological activity of TNF by binding to the soluble and transmembrane forms of TNF and inhibits the binding of TNF with its receptors. The structure of Infliximab is similar to that of naturally occurring antibodies. Eternarcept is a fusion protein made up of the extracellular domain of the p75 TNF receptor and the hinge and Fc domains of human IgG1.

Another example of an antagonist for a trimeric cytokine is given in WO 00/42073 where a monoclonal antibody against the trimeric cytokine TWEAK (TNFLS member 12) is disclosed. It is mentioned that the antibody can block the development of Graft-Versus-Host Disease.

However, a common technical problem that is encountered with the presently known binders and inhibitor products for trimeric cytokines, is that upon formation of a 1:1 complex of the trimeric cytokine and the binder or inhibitor product, not all three receptor binding sites of the cytokine are effectively blocked, as at least one or two of the three receptor binding sites are left open. The open cytokine receptor binding site(s) may associate with a corresponding cell surface cytokine receptor and thereby, due to high local concentration of the receptors on the cell surface, initiate the further recruitment of cytokine receptors and mediate receptor signalling.

The problem is clearly illustrated by the TNF binder Eternacept, which is a bivalent molecule that forms a 1:1 complex with the TNF trimer. When Eternacept binds to TNF, only two out of three possible receptor binding sites are occupied by the Eternacept molecule, and the third receptor binding site is left open. This implies that the open TNF receptor binding site may associate with a cell surface TNF receptor and thereby, due to high local concentration of the receptors on the cell surface, initiate the further recruitment of TNF receptors and by that signal transduction. This problem is illustrated in Figure 2.

The same problem is seen with the TNF binder Infliximab. Each Infliximab molecule is only capable of binding one receptor binding site (subunit) of a given TNF molecule, hence leaving out two open receptor binding sites. These open receptor binding sites may subsequently associate with corresponding free receptors, resulting in further recruitment of TNF receptors and receptor signalling.

It has now been found by the present inventors, that the above technical problems may be overcome by the provision of a fusion protein (protomer) which is constructed as a fusion of a binder for trimeric cytokines to a trimerising domain. The fusion protein of the present invention is, as a trimeric protein, capable of forming a 1:1 complex with a trimeric cytokine and at the same time effectively binding all three receptor binding sites of the trimeric cytokines, whereby no receptor binding sites are left open, and is therefore able to inhibit docking of the cytokine to the cell surface. Thus, there is now provided a fusion protein which has the advantage of being capable of more effectively inhibiting trimeric cytokines as compared to presently known antagonist. One of the advantages of the fusion protein according to the invention is that when applied as a medicament, less amount of the fusion protein is required in order to obtain a therapeutic effect, as compared to the presently known binders for trimeric cytokines.

### ***Summary of the invention***

Accordingly, the invention relates in a first aspect to a trimeric polypeptide comprising three monomers, each of said monomers comprising a specific binding member capable of binding a trimeric cytokine, and each of said monomers comprising a trimerising domain.

In a further aspect there is provided a pharmaceutical composition comprising the trimeric polypeptide according to the invention.

In a still further aspect, the invention pertains to a method of treating a subject having a pathology mediated by tumour necrosis factor by administering an effective amount of the trimeric polypeptide according to the invention to the subject,

Finally, there is provided a method for the preparation of the trimeric polypeptide according to invention, and an assay method for detecting a trimeric polypeptide cytokine in a sample.

### ***Detailed disclosure of the invention***

In one aspect, the present invention provides trimeric binders which are capable of binding to trimeric cytokines in a manner such that upon binding, all receptor binding sites of the trimeric cytokine are substantially blocked and/or neutralised, and hence the potential biological activity of the trimeric cytokine is suppressed or blocked. Accordingly, there is provided a trimeric polypeptide comprising three monomers, wherein each of the monomers comprise a specific binding member which is capable of binding a trimeric cytokine, and wherein each of the monomers comprise a trimerising domain.

In the present context, the term "trimeric cytokine" refers to small proteins and fragments thereof, which are produced and secreted by a cell, and which elicit a specific response in a cell which has a receptor for that cytokine, e.g. by affecting the growth, division and/or function of the cell. The term "trimeric" is used herein to describe that the cytokines according to the invention occur as multi-subunit complexes containing three subunits, preferably three identical subunits (homotrimer).

Typical examples of such trimeric cytokines include Macrophage migration inhibitory factor (MIF) and cytokines within the tumour necrosis factor ligand super family (TNFLSF). Presently, as mentioned above, the TNF ligand super family has at least 17 recognised

ligands which all share a conserved trimeric C-terminal domain known as the "TNF homology domain" (THD), which is responsible for receptor binding. The sequence identity of this trimeric domain is approximately 20-30% between TNF family members. The THD is a 150 amino acid long sequence containing a conserved framework of aromatic and hydrophobic residues which is illustrated in Figure 2 in Bodmer et al. 2002, *TRENDS in Biochemical Sciences* 27(1), pp. 19-26. Accordingly, in the present context a "member of the Tumor necrosis factor ligand superfamily" is intended to mean a trimeric protein comprising the TNF homology domain, THD. Presently known trimeric cytokines within the TNF ligand super family are listed below in Table 1, together with synonyms and their corresponding Genbank ID (GenBank, National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov/Genbank>). The nomenclature for the TNF ligand superfamily as used herein follows the official TNF superfamily (TNFSF) nomenclature which may be found at <http://www.gene.ucl.ac.uk/nomenclature/genefamily/tnftop.html>.

**TABEL 1: TNF ligand superfamily**

Ligand symbol	Genbank ID	Synonyms
LTA	X01393	TNFSF1, TNFB, LT
TNF	X02910	TNFSF2, TNF-alpha, DIF
LTB	L11016	TNFSF3, TNFC, p33
TNFSF4	D90224	OX-40L, gp34, TXGP1
TNFSF5	X67878	CD40LG, IMD3, HIGM1, CD40L, hCD40L, TRAP, CD154, gp39
TNFSF6	U11821	FasL, APT1LG1
TNFSF7	L08096	CD70, CD27L, CD27LG
TNFSF8	L09753	CD30LG
TNFSF9	U03398	4-1BB-L
TNFSF10	U37518	TRAIL, Apo-2L, TL2
TNFSF11	AF013171	TRANCE, RANKL, OPGL, ODF
TNFSF12	AF030099	TWEAK, DR3LG, APO3L
TNFSF13	NM_003808	APRIL
TNFSF13B	AF136293	BAFF, THANK, BLYS, TALL-1, TALL1, TNFSF20
TNFSF14	AF036581	LIGHT, LTg, HVEM-L
TNFSF Fn14	-	Fibroblast growth factor-inducible immediate-early response protein 14, FGF-inducible 14, Tweak-receptor, TweakR
TNFSF15	AF039390	TL1, VEGI
TNFSF18	AF125303	AITRL TL6 hGITRL

Accordingly, in one useful embodiment of the invention the trimeric polypeptide according to the invention is capable of binding a trimeric cytokine which is a member of the Tumor



necrosis factor ligand superfamily selected from LTA; TNF ;LTB; TNFSF4; TNFSF5; TNFSF6; TNFSF7; TNFSF8; TNFSF9; TNFSF10; TNFSF11; TNFSF12; TNFSF13; TNFSF13B; TNFSF14; TNFRSF Fn14; TNFSF15; and TNFSF18.

The term "specific binding member", as used herein, refers to a member of a pair of molecules which have binding specificity for one another. The members of a specific binding pair may be naturally derived or wholly or partially synthetically produced. One member of the pair of molecules has an area on its surface, or a cavity, which specifically binds to and is therefore complementary to a particular spatial and polar organisation of the other member of the pair of molecules. Thus the members of the pair have the property of binding specifically to each other. Examples of types of specific binding pairs are antigen-antibody, biotin-avidin, hormone-hormone receptor, ligand-ligand receptor, enzyme-substrate. Other examples of specific binding pairs include, carbohydrates and lectins, complementary nucleotide sequences (including probe and capture nucleic acid sequences used in DNA hybridization assays to detect a target nucleic acid sequence), complementary peptide sequences including those formed by recombinant methods, effector and receptor molecules, enzyme cofactors and enzymes, enzyme inhibitors and enzymes, and the like. Furthermore, specific binding pairs can include members that are analogs or fragments of the original specific binding member.

In one useful embodiment of the present invention, the specific binding member, which is capable of binding a trimeric cytokine, is a polypeptide derived from a member of the Tumor necrosis factor receptor superfamily. Presently, at least 29 receptors within the Tumor necrosis factor superfamily are known, and listed below in Table 2 together with their synonyms and their corresponding Genbank ID (GenBank, National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov/Genbank>). The nomenclature for the members of the TNF receptor superfamily as used herein follows the official TNF superfamily (TNFSF) nomenclature which may be found at <http://www.gene.ucl.ac.uk/nomenclature/genefamily/tnftop.html>.

**TABEL 2: TNF receptor superfamily**

Receptor Symbol	Genbank ID	Synonyms
TNFRSF1A	M75866	p55-R, CD120a, TNF-R-I p55, TNF-R, TNFR1, TNFAR, TNF-R55, p55TNFR, TNFR60
TNFRSF1B	M32315	CD120b, p75, TNF-R, TNF-R-II, TNFR80, TNFR2, TNF-R75, TNFBR, p75TNFR
LTBR	L04270	TNFRSF3, TNFR2-RP, CD18, TNFR-RP, TNFCR, TNF-R-III
TNFRSF4	X75962	OX40, ACT35, TXGP1L
TNFRSF5	X60592	p50, Bp50, CD40
TNFRSF6	M67454	FAS, CD95, APO-1, APT1
TNFRSF6B	AF104419	DcR3, M68, TR6, HGNC:15888, NHL, DKFZP434C013, KIAA1088, bK3184A7.3, C20orf41
TNFRSF7	M63928	Tp55, S152, CD27
TNFRSF8	M83554	Ki-1, D1S166E, CD30
TNFRSF9	L12964	4-1BB, CD137, ILA
TNFRSF10A	U90875	DR4, Apo2, TRAILR-1
TNFRSF10B	AF012628	DR5, KILLER, TRICK2A, TRAIL-R2, TRICKB
TNFRSF10C	AF012538	DcR1, TRAILR3, LIT, TRID
TNFRSF10D	AF029761	DcR2, TRUNDD, TRAILR4
TNFRSF11A	AF018253	RANK
TNFRSF11B	U94332	OPG, OCIF, TR1
TNFRSF12	U72763	DR3, TRAMP, WSL-1, LARD, WSL-LR, DDR3, TR3, APO-3
TNFRSF12L	-	DR3L
TNFRSF13B	AF023614	TACI
TNFRSF13C	AF373846	BAFFR
TNFRSF14	U70321	HVEM, ATAR, TR2, LIGHTR, HVEA
NGFR	M14764	TNFRSF16, p75NTR
TNFRSF17	Z29574	BCMA, TNFRSF13
TNFRSF18	AF125304	AITR, GITR
TNFRSF19	AB040434	TAJ-alpha, TROY, TAJ, TRADE
TNFRSF19L	AF319553	FLJ14993, RELT
TNFRSF21	AF068868	DR6
TNFRSF22	-	SOBa, Tnfrh2, 2810028K06Rik
TNFRSF23	-	mSOB, Tnfrh1

Thus, in useful embodiments the specific binding member derived from a receptor within the Tumor necrosis factor superfamily is selected from the receptors TNFRSF1A, TNFRSF1B, LTBR, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF11A, TNFRSF11B, TNFRSF12, TNFRSF12L, TNFRSF13B, TNFRSF13C, TNFRSF14, NGFR, TNFRSF17, TNFRSF18, TNFRSF19, TNFRSF19L, TNFRSF21, TNFRSF22, and TNFRSF23. However, it is also contemplated that further useful receptors may be identified within this protein family, and hence further functional specific binding members.

In a useful embodiment, the specific binding member is derived from the TNF receptors TNFRSF1A (TNF $\alpha$ , p55 receptor) and/or TNFRSF1B (TNF $\beta$ , p75 TNF receptor). Especially the TNFRSF1B receptor and subunits thereof has proven to be effective for specifically binding and neutralising TNF. As an example, the TNF binding protein Eternacept is a fusion protein made up of the extracellular domain of the TNFRSF1B receptor, i.e. a subunit of the TNFRSF1B receptor. Accordingly, in specific embodiments, the trimeric polypeptide according to invention includes a specific binding member which is a subunit or fragment of the TNFRSF1B receptor (TNF $\beta$ ), such as the TNFRSF1B receptor fragments disclosed in US 5,712,155. Thus, in useful embodiments the specific binding member is a polypeptide comprising an amino acid sequence selected from TNFRSF1B 1-235 (SEQ ID NO:76), TNFRSF1B 1-185 (SEQ ID NO:77), TNFRSF1B 1-163 (SEQ ID NO:78) and TNFRSF1B 1-142 (SEQ ID NO:79).

In further useful embodiments, as disclosed in following examples, the specific binding member may be one or more fragments of the TNFRSF1B receptor (TNF $\beta$ ) and combinations of such fragments, such as a polypeptide comprising an amino acid sequence encoded by a DNA sequence selected from TNFRSF1B D1D2 (SEQ ID NO:13), TNFRSF1B D1D2, 1/6 (SEQ ID NO:15), TNFRSF1B D1D2 1/4 (SEQ ID NO:17), TNF $\beta$  D1D2, 1/3 (SEQ ID NO:19), TNFRSF1B D1D2, 1/2 (SEQ ID NO:21), TNFRSF1B D1D4 (SEQ ID NO:23), TNFRSF1B D2 (SEQ ID NO:25), TNFRSF1B D2, 1/6 (SEQ ID NO:26), TNFRSF1B D2, 1/4 (SEQ ID NO:27), TNFRSF1B D2, 1/3 (SEQ ID NO:28), TNFRSF1B D2, 1/2 (SEQ ID NO:29) and TNFRSF1B D2D4 (SEQ ID NO:30).

In accordance with the invention, the specific binding member capable of binding a trimeric cytokine may also be an antibody or an antibody fragment. In the present context, the term "antibody" is used to describe an immunoglobulin whether natural or partly or wholly synthetically produced. As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any specific binding member or substance having a binding domain with the required trimeric cytokine specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain,

whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. The term also covers any polypeptide or protein having a binding domain which is, or is homologous to, an antibody binding domain, e.g. antibody mimics. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses; fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies.

US 6,451,983 B2 describes antibodies for human tumour necrosis factor which are i.a. capable of neutralising the biological activity of TNF. In particular, US 6,451,983 B2 provides antibodies and fragments thereof which are capable of binding to mature human TNF (SEQ ID NO:80) within certain specific topographic regions. Accordingly, in useful embodiments of the present invention, the specific binding member is an antibody or fragment thereof which is capable of binding mature human TNF (SEQ ID NO:80) in at least one region selected from the regions consisting of amino acid residues 1-18, 1-20, 1-26, 1-30, 12-22, 22-31, 22-40, 36-45, 49-97, 49-98, 56-79, 58-65, 69-97, 70-87, 76-90, 96-105, 105-128, 108-128, 110-127, 115-125, 117-128, 132-157, 135-155, 136-153, 138-149, 141-153 and 146-157.

US 6,451,983 B2 further provides specific monoclonal antibodies which may be useful in the present invention. Accordingly, the specific binding member capable of binding human TNF may be an antibody or antibody fragment selected from MAb 1 (ECACC 89080301), MAb 21 (ECACC 90012432), MAb 25 (ECACC 89121401), MAb 32 (ECACC 89080302), MAb 37 (ECACC 89080303), MAb 42 (ECACC 89080304), MAb 47 (ECACC 89121402), MAb 53 (ECACC 90012433) and MAb 54 (ECACC 89083103), wherein ECACC refers to European Collection of Animal Cell Cultures.

Further examples of useful antibodies include the humanised antibody D2E7 as disclosed in US 6,090,382 and the humanised antibody fragment CDP 870 as disclosed in WO/00194585.

In specific embodiments, the specific binding members of the present invention may comprise antibody mimics or artificial antibodies such as e.g. a protein having the scaffold structure of C-type lectin-like domains (CTLD) as disclosed in WO/0248189.

An important aspect of the present invention is that the monomers of the trimeric polypeptide, in addition to the specific binding member, further comprise a trimerising domain. In the present context, the term "trimerising domain" is a peptide, a protein or part of a protein which is capable of interacting with other, similar or identical trimerising domains. The interaction is of the type that produces trimeric proteins or polypeptides. Such an interaction may be caused by covalent bonds between the components of the trimerising domains as well as by hydrogen bond forces, hydrophobic forces, van der Waals forces and salt bridges.

One example of a trimerising domain is disclosed in WO 95/31540, which describes polypeptides comprising a collectin neck region. The amino acid sequence constituting the collectin neck region may be attached to any polypeptide of choice. Trimers can then be made under appropriate conditions with three polypeptides comprising the collectin neck region amino acid sequence.

In a presently preferred embodiment, the trimerising domain is derived from tetranectin, and more specifically comprises the tetranectin trimerising structural element (hereafter termed TTSE) which is described in detail in WO 98/56906. The amino acid sequence of TTSE is shown in SEQ ID NO 81. The trimerising effect of TTSE is caused by a coiled coil structure which interacts with the coiled coil structure of two other TTSEs to form a triple alpha helical coiled coil trimer which is exceptionally stable even at relatively high temperatures. The term TTSE is also intended to embrace variants of a TTSE of a naturally occurring member of the tetranectin family of proteins, variants which have been modified in the amino acid sequence without adversely affecting, to any substantial degree, the capability of the TTSE to form alpha helical coiled coil trimers. Thus, the trimeric polypeptide according to the invention may comprise a TTSE as a trimerising domain, which comprises a sequence having at least 68% amino acid sequence identity with the sequence of SEQ ID NO 81, such as at least 75%, including at least

87%, such as at least 92%. In accordance herewith, the cystein residue No. 50 of the TTSE (SEQ ID NO:81) may advantageously be mutagenised to serine, threonine, methionine or to any other amino acid residue in order to avoid formation of an unwanted inter-chain disulphide bridge, which could lead to unwanted multimerisation.

In a further embodiment, the TTSE trimerising domain (SEQ ID NO 81) may be modified by (i) the incorporation of polyhistidine sequence and/or a cleavage site for the Blood Coagulating Factor X<sub>a</sub>, (ii) replacing Cys 50 with Ser, and (iii) by including a C-terminal KGS sequence.

Several examples of different trimeric polypeptides in accordance with the invention are provided in the following in examples, in which the above TTSE trimerising domain has been applied.

In accordance with the invention, the specific binding member may either be linked to the N- or the C-terminal amino acid residue of the trimerising domain. However, it is also envisaged that in certain embodiments it may be advantageous to link a specific binding member to both the N-terminal and the C-terminal of the trimerising domain of the monomer, and thereby providing a trimeric polypeptide comprising six specific binding members capable of binding a trimeric cytokine.

It will be appreciated that a flexible molecular linker optionally may be interposed between, and covalently join, the specific binding member and the trimerising domain. Preferably, the linker is a polypeptide sequence of about 1-20 amino acid residues, such as about 2-10 amino acid residues, including 3-7 amino acid residues. In useful embodiments the linker is essentially non-immunogenic, not prone to proteolytic cleavage and does not comprise amino acid residues which are known to interact with other residues (e.g. cystein residues).

The trimeric polypeptide of the present invention may be expressed in any suitable standard protein expression system by culturing a host transformed with a vector encoding the trimeric polypeptide under such conditions that the trimeric polypeptide is expressed.

Preferably, the expression system is a system from which the desired protein may readily be isolated and refolded *in vitro*. As a general matter, prokaryotic expression systems are preferred since high yields of protein can be obtained and efficient purification and refolding strategies are available. Thus, it is well within the abilities and discretion of the skilled artisan, without undue experimentation, to choose an appropriate or favourite expression system. Similarly, once the primary amino acid sequence for the trimeric polypeptide of the present invention is chosen, one of ordinary skill in the art can easily design appropriate recombinant DNA constructs which will encode the desired proteins, taking into consideration such factors as codon biases in the chosen host, the need for secretion signal sequences in the host, the introduction of proteinase cleavage sites within the signal sequence, and the like. These recombinant DNA constructs may be inserted in-frame into any of a number of expression vectors appropriate to the chosen host. The choice of an appropriate or favourite expression vector is, again, a matter well within the ability and discretion of the skilled practitioner. Preferably, the expression vector will include a strong promoter to drive expression of the recombinant constructs. Finally, the trimeric polypeptide may be isolated using suitable standard procedures well known in the art, and optionally subjected to further processing such as e.g. lyophilization.

The trimeric polypeptide according to the invention may be used for the preparation of a pharmaceutical composition by any suitable method well known in the art. The composition may together with the trimeric polypeptide, comprise one or more acceptable carriers therefore, and optionally other therapeutic ingredients. The carriers must be acceptable in the sense of being compatible with the other ingredients and not deleterious to the recipient thereof. In general, methods for the preparation of pharmaceutical compositions include the step of bringing into association the active ingredient and a carrier.

The therapeutic application of the present invention comprises the treatment of a disease or disorder in an animal, by administering a therapeutically effective amount of the trimeric polypeptide according to the invention to the animal in need thereof. As mentioned above, trimeric cytokines, and in particular trimeric cytokines of the TNF ligand super family, are known to be pleiotropic polypeptides that act as intercellular signal transduction molecules

by i.a. controlling and coordinating immune and inflammatory responses. Thus, it will be appreciated that the trimeric polypeptide of the present invention may be applicable for the treatment of wide range of disorders and diseases mediated by trimeric cytokines, including inflammatory diseases, autoimmune disorders, immune disorders, infections, malignancies, and neurogenerative diseases. In one useful embodiment the disease is a pathology mediated by tumor necrosis factor, such as rheumatoid arthritis, psoriasis and Crohn's disease.

The trimeric polypeptide of the present invention may be directly administered to the animal by any suitable technique, including parenterally, and can be administered locally or systemically. The specific route of administration depends, e.g., on the medical history of the animal. Examples of parenteral administration include subcutaneous, intramuscular, intravenous, intraarterial, and intraperitoneal administration.

The animals potentially treatable by the trimeric fusion protein herein include mammals such as a human.

Finally, the present invention provides an assay method for detecting a trimeric polypeptide cytokine in a sample which comprises (i) contacting the sample with a trimeric polypeptide according to the invention, and (ii) detecting the binding of the trimeric polypeptide to the trimeric cytokine.

The invention will now be described by way of illustration in the following non-limiting examples and figures.

### ***Description of the figures***

**Figure 1** shows the binding of a trimeric cytokine within the Tumour Necrosis Factor Super Family to a corresponding cell bound monomeric receptor, which initiates the further recruitment of two more cytokine receptors resulting in the formation of a hexameric cytokine-receptor signalling unit.



**Figure 2** shows the binding of a bivalent binder to a trimeric cytokine within the Tumour Necrosis Factor Super Family (TNFSF), which forms a 1:1 complex with the trimeric cytokine. When the bivalent binder binds to the trimeric TNFSF cytokine, only two out of three possible receptor binding sites are occupied by the bivalent binder molecule, and the third receptor binding site is left open. This implies that the open trimeric cytokine receptor binding site may associate with a cell surface trimeric cytokine receptor and thereby, due to high local concentration of the receptors on the cell surface, initiate the further recruitment of trimeric receptors and thereby signal transduction.

**Figure 3** shows a binder for trimeric cytokines which is capable of forming a 1:1 complex with a trimeric cytokine and at the same time effectively binding all three receptor binding sites of the trimeric cytokine, whereby no receptor binding sites are left open. The docking of the cytokine to the cell surface is blocked, and hence no signal transduction will occur.

## ***Examples***

### **EXAMPLE 1**

#### **Design and construction of trip *E. coli* expression plasmids and phagemids for the production of trimeric cytokine binders**

##### **Phagemids**

The phagemid, psktripB, is constructed by ligation of the Sfi I and Not I restricted DNA fragment sktripB amplified from the expression plasmid pTtripb with the oligonucleotide primers C-sfikpn-TRI (SEQ ID NO:1) and N-sfikpn-TRI (SEQ ID NO:2) into a Sfi I and Not I pre-cut vector, pCANTAB 5E supplied by Amersham Pharmacia Biotech (code no. 27-9401-01) using standard procedures. The nucleotide sequences of the sktripB inserts is given as SEQ ID NO:3.

The phagemid, pskl10tripB, is constructed by ligation of the Sfi I and Not I restricted DNA fragment skl10tripB amplified from the expression plasmid pTtripb with the oligonucleotide primers C-sfikpn-TRI (SEQ ID NO:1) and N-sfikpn-l10TRI (SEQ ID NO:4) into a Sfi I and

Not I pre-cut vector, pCANTAB 5E supplied by Amersham Pharmacia Biotech (code no. 27-9401-01) using standard procedures. The nucleotide sequence of the skl10tripB inserts is given as SEQ ID NO: 5.

#### Expression plasmids

The expression plasmid pT7H6-bktripB, is constructed by ligation of the BamH I and Hind I restricted DNA fragment bktripB, amplified from the expression plasmid pTtripb with the oligonucleotide primers C-bamkpn-TRI (SEQ ID NO:6) and N-bamkpn-TRI (SEQ ID NO: 7), in to the BamH I and Hind I restricted expression plasmid pT7H6 using standard procedures. The nucleotide sequence of bktripB is given as SEQ ID NO:8.

The expression plasmid pT7H6-bkl10tripB, is constructed by ligation of the BamH I and Hind I restricted DNA fragment bkl10tripB, amplified from the expression plasmid pTtripb with the oligonucleotide primers C-bamkpn-TRI (SEQ ID NO: 6) and N-bamkpn-I10TRI (SEQ ID NO:9), in to the BamH I and Hind I restricted expression plasmid pT7H6 using standard procedures. The nucleotide sequence of bkltripB is given as SEQ ID NO:10.

## **Example 2**

### **Design and construction of a trimeric TNF binder using TNFRII fragments**

Fragments and subunits of the TNF receptor TNFRSF1B (TNFRII) is used for the construction of trimeric TNF binders based on the trimerising domain TripB derived from tetranectin. The design and cloning is outlined in the following:

#### Cloning of TNFRSF1B fragments into Phagemid vectors

The Phagemid vector pD1D2tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D2kpn-fo (SEQ ID NO: 12)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2, is given as SEQ ID NO:13.

The Phagemid vector pD1D2,1/6tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D1/6 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) D1/6kpn-fo (SEQ ID NO: 14)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2,1/6, is given as SEQ ID NO:15.

The Phagemid vector pD1D2,1/4tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D1/4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D1/4kpn-fo (SEQ ID NO: 16)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2,1/4, is given as SEQ ID NO:17.

The Phagemid vector pD1D2,1/3tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D1/3 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D1/3kpn-fo (SEQ ID NO: 18)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2,1/3, is given as SEQ ID NO:19.

The Phagemid vector pD1D2,1/2tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D1/2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D1/2kpn-fo (SEQ ID NO: 20)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2,1/2, is given as SEQ ID NO:21.

The Phagemid vector pD1D4tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1)

(with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D4kpn-fo (SEQ ID NO: 22)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D4, is given as SEQ ID NO:23.

The Phagemid vector pD1D2I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D2kpn-fo (SEQ ID NO: 12)) into a Sfi I and Kpn I cut vector, pskI10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2, is given as SEQ ID NO:13.

The Phagemid vector pD1D2,1/6I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D1/6 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D1/6kpn-fo (SEQ ID NO: 14)) into a Sfi I and Kpn I cut vector, pskI10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2,1/6, is given as SEQ ID NO:15.

The Phagemid vector pD1D2,1/4I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D1/4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D1/4kpn-fo (SEQ ID NO: 16)) into a Sfi I and Kpn I cut vector, pskI10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2,1/4, is given as SEQ ID NO:17.

The Phagemid vector pD1D2,1/3I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D1/3 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev: (SEQ ID NO: 11) and D1/3kpn-fo (SEQ ID NO: 18)) into a Sfi I and Kpn I cut vector, pskI10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2,1/4, is given as SEQ ID NO:19.

The Phagemid vector pD1D2,1/2I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D1/2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D1/2kpn-fo (SEQ ID NO: 20)) into a Sfi I and Kpn I cut vector, pskI10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D2,1/2, is given as SEQ ID NO:21.

The Phagemid vector pD1D4I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1D4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D1-rev (SEQ ID NO: 11) and D4kpn-fo (SEQ ID NO: 22)) into a Sfi I and Kpn I cut vector, pskI10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D1D4, is given as SEQ ID NO:23.

The Phagemid vector pD2tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D2kpn-fo (SEQ ID NO: 12)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2, is given as SEQ ID NO:25.

The Phagemid vector pD2,1/6tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1/6 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D1/6kpn-fo (SEQ ID NO: 14)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2,1/6, is given as SEQ ID NO:26.

The Phagemid vector pD2,1/4tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1/4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D1/4kpn-fo (SEQ ID NO:

16)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2,1/4, is given as SEQ ID NO:27.

The Phagemid vector pD2,1/3tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1/3 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D1/3kpn-fo (SEQ ID NO: 18)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2,1/3, is given as SEQ ID NO:28.

The Phagemid vector pD21/2tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1/2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D1/2kpn-fo (SEQ ID NO: 20)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2,1/2, is given as SEQ ID NO:29.

The Phagemid vector pD2D4tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D2D4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D4kpn-fo (SEQ ID NO: 22)) into a Sfi I and Kpn I cut vector, psktripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2D4, is given as SEQ ID NO:30.

The Phagemid vector pD2I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D2kpn-fo (SEQ ID NO: 12)) into a Sfi I and Kpn I cut vector, pskI10tripB using standard procedures. Outlines of the resulting nucleotide sequence of D2, is given as SEQ ID NO:25.

The Phagemid vector pD2,1/6I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1/6 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D1/6kpn-fo (SEQ ID NO: 14)) into a Sfi I and Kpn I cut vector, pskl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2,1/6, is given as SEQ ID NO:26.

The Phagemid vector pD2,1/4I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1/4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D1/4kpn-fo (SEQ ID NO: 16)) into a Sfi I and Kpn I cut vector, pskl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2,1/4, is given as SEQ ID NO:27.

The Phagemid vector pD2,1/3I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1/3 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D1/3kpn-fo (SEQ ID NO: 18)) into a Sfi I and Kpn I cut vector, pskl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2,1/3, is given as SEQ ID NO:28.

The Phagemid vector pD2,1/2I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D1/2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D1/2kpn-fo (SEQ ID NO: 20)) into a Sfi I and Kpn I cut vector, pskl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2,1/2, is given as SEQ ID NO:29.

The Phagemid vector pD2D4I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment D2D4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers D2-rev (SEQ ID NO: 24) and D4kpn-fo (SEQ ID NO: 22))

into a Sfi I and Kpn I cut vector, pskl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of D2D4, is given as SEQ ID NO:30.

**Cloning of TNFRSF1B (TNFRII) receptor fragments into expression vectors**

The expression vector pT7H6FXD1D2tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D2kpn-fo (SEQ ID NO: 12)) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2, is given as SEQ ID NO:32.

The expression vector pT7H6FXD1D2,1/6tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/6 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D1/6kpn-fo (SEQ ID NO: 14)) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2,1/6, is given as SEQ ID NO:33.

The expression vector pT7H6FXD1D2,1/4tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D1/4kpn-fo (SEQ ID NO: 16)) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2,1/4, is given as SEQ ID NO:34.

The expression vector pT7H6FXD1D2,1/3tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/3 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and



D1/3kpn-fo (SEQ ID NO: 18)) into a BamH I and Kpn I cut vector, pT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2,1/3, is given as SEQ ID NO:35.

The expression vector pT7H6FXD1D2,1/2tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D1/2kpn-fo (SEQ ID NO: 20)) into a BamH I and Kpn I cut vector, pT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2,1/2, is given as SEQ ID NO:36.

The expression vector pT7H6FXD1D4tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment FXD1D4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D4kpn-fo (SEQ ID NO: 22)) into a BamH I and Kpn I cut vector, pT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D4, is given as SEQ ID NO:37.

The expression vector pT7H6FXD2tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D2kpn-fo (SEQ ID NO: 12)) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2, is given as SEQ ID NO:39.

The expression vector pT7H6FXD2,1/6tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1/6 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) bamD2-rev (SEQ ID NO: 38) and D1/6kpn-fo (SEQ ID NO: 14)) into a BamH I and Kpn I cut vector, PT7H6-

bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2,1/6, is given as SEQ ID NO:40.

The expression vector pT7H6FXD2,1/4tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D1/4kpn-fo (SEQ ID NO: 16)) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2,1/4, is given as SEQ ID NO:41.

The expression vector pT7H6FXD2,1/3tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/3 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D1/3kpn-fo (SEQ ID NO: 18)) into a BamH I and Kpn I cut vector, pT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2,1/3, is given as SEQ ID NO:42.

The expression vector pT7H6FXD2,1/2tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1/2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D1/2kpn-fo (SEQ ID NO: 20)) into a BamH I and Kpn I cut vector, pT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2,1/2, is given as SEQ ID NO:43.

The expression vector pT7H6FXD2D4tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment FXD2D4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D4kpn-fo (SEQ ID NO:

22)) into a BamH I and Kpn I cut vector, pT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2D4, is given as SEQ ID NO:44.

The expression vector pT7H6FXD1D2I10tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D2kpn-fo (SEQ ID NO: 12)) into a BamH I and Kpn I cut vector, PT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2, is given as SEQ ID NO:32.

The expression vector pT7H6FXD1D2,1/6I10tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/6 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D1/6kpn-fo (SEQ ID NO: 14)) into a BamH I and Kpn I cut vector, PT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2,1/6, is given as SEQ ID NO:33.

The expression vector pT7H6FXD1D2.1/4I10tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D1/4kpn-fo (SEQ ID NO: 16)) into a BamH I and Kpn I cut vector, PT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2,1/4, is given as SEQ ID NO:34.

The expression vector pT7H6FXD1D2,1/3I10tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/3 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D1/3kpn-fo (SEQ ID NO: 18)) into a BamH I and Kpn I cut vector, pT7H6-bkl10tripB using

standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2,1/3, is given as SEQ ID NO:35.

The expression vector pT7H6FXD1D2,1/2I10tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D1/2kpn-fo (SEQ ID NO: 20)) into a BamH I and Kpn I cut vector, pT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D2,1/2, is given as SEQ ID NO:36.

The expression vector pT7H6FXD1D4I10tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment FXD1D4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD1-rev (SEQ ID NO: 31) and D4kpn-fo (SEQ ID NO: 22)) into a BamH I and Kpn I cut vector, pT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD1D4, is given as SEQ ID NO:37.

The expression vector pT7H6FXD2I10tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D2kpn-fo (SEQ ID NO: 12)) into a BamH I and Kpn I cut vector, PT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2, is given as SEQ ID NO:39.

The expression vector pT7H6FXD2,1/6I10tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1/6 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D1/6kpn-fo (SEQ ID NO: 14)) into a BamH I and Kpn I cut vector, PT7H6-bkl10tripB using standard

procedures. Outlines of the resulting the nucleotide sequence of FXD2,1/6, is given as SEQ ID NO:40.

The expression vector pT7H6FXD2,1/410tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D1/4kpn-fo (SEQ ID NO: 16)) into a BamH I and Kpn I cut vector, PT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2,1/4, is given as SEQ ID NO:41.

The expression vector pT7H6FXD2,1/310tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1D1/3 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D1/3kpn-fo (SEQ ID NO: 18)) into a BamH I and Kpn I cut vector, pT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2,1/3, is given as SEQ ID NO:42.

The expression vector pT7H6FXD2,1/210tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXD1/2 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D1/2kpn-fo (SEQ ID NO: 20)) into a BamH I and Kpn I cut vector, pT7H6-bkl10tripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXD2,1/2, is given as SEQ ID NO:43.

The expression vector pT7H6FXD2D410tripB, is constructed by ligation of the Sfi I and Kpn I restricted DNA fragment FXD2D4 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bamD2-rev (SEQ ID NO: 38) and D4kpn-fo (SEQ ID NO: 22)) into a BamH I and Kpn I cut vector, pT7H6-bkl10tripB using standard

procedures. Outlines of the resulting the nucleotide sequence of FXD2D4, is given as SEQ ID NO:44.

### **Example 3**

#### **Design, construction and production of a trimeric TNF binder using D2E7 antibody fragments.**

Single chain antibody variable region fragments (scFv) of the humanised antibody D2E7 is selected as binders in the construction of a trimeric polypeptide capable of binding TNF. The trimeric binder may be constructed by amplifying the VH and VL domains of D2E7, based on the sequence disclosed in US 6,090,382.

#### **Design and construction of trimeric D2E7 scFv with a Gly,Thr linker between N-terminal scFv and C-terminal trimerising domain**

The expression vector pT7H6FXD2E7tripB is constructed in two steps. The first step is the construction of pT7H6FX (D2E7VH)tripB. This vector is constructed by ligation of the Bgl II and Kpn I restricted DNA fragment FX(D2E7VH) amplified from DNA containing the sequence of D2E7 (with the oligonucleotide primers FXVH (SEQ ID NO: 45) and VHBamKpn (SEQ ID NO: 46)) into a BamH I Kpn I cut pT7H6bktripB vector. The second step is the construction of pT7H6FXD2E7tripB by ligation of the BamH I Kpn I restricted DNA fragment G4SVL amplified from DNA containing the sequence of D2E7 (with the oligonucleotide primers G4SVL (SEQ ID NO: 47) and VLkpn (SEQ ID NO: 48)) into a BamH I Kpn I cut pT7H6FX(D2E7VH)tripB. The nucleotide sequence of FXD2E7, is given as SEQ ID NO:49.

#### **Design and construction of trimeric D2E7 scFv with a Gly, Gly, Gly, Gly, Ser, Gly, Thr linker between N-terminal scFv and C-terminal trimerising domain**

The expression vector pT7H6FXD2E7G4StripB is constructed in two steps. The first step is the construction of pT7H6FX(D2E7VH)tripB by ligation of the Bgl II and Kpn I restricted

DNA fragment FX(D7E2VH) amplified from DNA containing the sequence of D2E7 (with the oligonucleotide primers FXVH (SEQ ID NO: 45) and VHBamKpn (SEQ ID NO: 46)) into a BamH I Kpn I cut pT7H6bktripB vector. The second step is the construction of pT7H6FXD2E7tripB by ligation of the BamH I Kpn I restricted DNA fragment G4SVL amplified from DNA containing the sequence of D2E7 (with the oligonucleotide primers G4SVL (SEQ ID NO: 47) and VLG4Skpn (SEQ ID NO: 50)) into a BamH I Kpn I cut pT7H6FX(D2E7VH)tripB. The resulting nucleotide sequence of FXD2E7G4S, is given as SEQ ID NO:51.

**Design and construction of trimeric D2E7 scFv with Gly, Ser linker between the N-terminal trimerising domain and the C-terminal scFv**

The Expression vector pT76HFXTripAGSD2E7, is constructed by ligation of the Bcl I and Hind III restricted DNA fragment amplified from pT7H6FXD2E7G4StripB (with the oligonucleotide primers bclVH (SEQ ID NO:52) and VLhind (SEQ ID: 53)) into a BamH I and Hind III cut vector, pT76HFXtripa (Loretsen, RH. et al, Biochem J.;347:83-87,2000) using standard procedures. The resulting nucleotide sequence of GSD2E7, is given as SEQ ID NO:54.

**Design and construction of trimeric D2E7 scFv with Gly, Ser, Gly, Gly, Gly, Gly, Ser linker between the N-terminal trimerising domain and the C-terminal scFv**

The Expression vector pT76HFXTripAG4SD2E7, is constructed by ligation of the Bcl I and Hind III restricted DNA fragment amplified from pT7H6FXD2E7G4StripB (with the oligonucleotide primers bclG4SVH (SEQ ID NO:55) and VLhind (SEQ ID: 53)) into a BamH I and Hind III cut vector, pT76HFXtripa (Loretsen, RH. et al, Biochem J.;347:83-87,2000) using standard procedures. The resulting nucleotide sequence of G4SD2E7, is given as SEQ ID NO:56.

To prepare the above fusion proteins H6FXD2E7tripB, H6FXD2E7G4StripB,

H6FXTripAD2D7TripA and H6FXTripAG4SD2E7, the plasmids pT7H6FXD2E7tripB, pT7H6FXD2E7G4StripB, pT7H6FXTripAD2D7TripA and pT7H6FXTripAG4SD2E7 is grown in small scale (1 litre; 2xTY medium, 5 mM MgSO<sub>4</sub> and 100 µg ampicillin) in *E. coli* BL21 cells, as described by Studier et al. (1990). Exponentially growing cultures at 37°C are at OD<sub>600</sub> 0.8 infected with bacteriophage lambda CE6 at a multiplicity of approximately 5. Cultures are grown at 37°C for another three hours and the cells harvested by centrifugation. Cells are re-suspended in 50 ml of 0.5 M NaCl, 50 mM Tris-HCl pH 8, and 1 mM EDTA pH 8. Phenol (50 ml adjusted to pH 8) is added to each and the mixtures are sonicated to extract total protein. After clarification by centrifugation (25 minutes at 10,000 g) crude protein fractions are precipitated from the phenol phases by addition of 2.5 volumes of ethanol and centrifugation. Protein pellets are dissolved in a buffer (15-25 ml) containing 6 M guanidinium chloride, 50 mM Tris-HCl pH 8 and 0.1 M dithioerythritol. Following gel filtration on Sephadex G-25 (Pharmacia, Sweden) into 8 M Urea, 1M NaCl, 50 mM Tris-HCl pH 8 and 10 mM 2-mercaptoethanol, the crude protein preparations are applied to Ni activated NTA-agarose columns (75 ml column volume) for purification (Hochuli et al., 1988). Washing buffer (6 M guanidine-HCl, 50 mM Tris-HCl pH 8 and 10 mM 2-mercaptoethanol) is then flowed through the columns until stable baselines are obtained.

Virtually pure fusion proteins are then eluted by applying a pH gradient to each column (1000 ml gradient in 8 M urea and 10 mM 2-mercaptoethanol obtained by linear (per volume) mixing of solutions containing 50 mM sodium di-hydrogenphosphate (pH 5 buffer) and 50 mM di-sodium hydrogenphosphate (pH 8 buffer).

In preparation for in vitro refolding by the method of Thøgersen et al. (WO 94/18227) 20 mg of each purified fusion protein are mixed in suspensions in refolding "buffer B" (described below) with aliquots of suspensions of Ni<sup>2+</sup> activated NTA-agarose matrix sufficient to generate columns of about 75 ml packed bed volume. Each fusion protein is then subjected to the iterative refolding procedure as described for plasminogen kringle 4 in the Thøgersen et al. patent application (WO 94/18227), except that refolding of the scFv containing fusion proteins is carried out at 10°C using a buffer containing 0.5 M NaCl, 50 mM Tris-HCl pH 8, 2 mM glutathione and 0.2 mM oxidized glutathione as "buffer A" and a



buffer containing 8 M urea, 1 M NaCl, 50 mM Tris-HCl pH 8 and 2 mM glutathione as "buffer B".

After completion of the refolding procedure each column is washed with 300 ml buffer containing 0.5 M NaCl and 50 mM Tris-HCl pH 8 to wash away glutathione. The refolded fraction of each protein is then eluted from the NTA-agarose in 20 mM EDTA, 0.5 M NaCl and 50 mM Tris-HCl pH 8 to the elution buffer. After addition of solid urea to achieve a final concentration of about 8 M to each protein sample and dilution or dialysis to reduce NaCl concentrations to below 5 mM, final purification of each correctly folded fusion protein product is then accomplished by ion exchange chromatography (S-Sepharose, Pharmacia, 1,6 (i.d.) by 90 centimeter column in a buffer containing 8 M urea, 5 mM Tris-HCl (from 1 M stock solution at pH 8) and 25 mM sodium acetate (from 1 M stock solution at pH 5), eluted at 2 ml/min). After dialysis against aqueous buffers (e.g. phosphate buffered saline) each pure and correctly refolded fusion protein is recovered in yields of 2-6 mg per litre of culture grown.

#### **Example 4**

##### **Design and construction of a trimeric APRIL binder using TNFRSF13B receptor fragments**

The APRIL receptor TNFRSF13B is used for the construction of trimeric binders against the trimeric cytokine APRIL, and based on the trimerising domain TripB derived from tetranectin. The design and cloning of such binders with different linkers is outlined in the following:

##### **Cloning of TNFRSF13B6**

The expression vector pT7H6FXTNFRSF13B6tripB, is constructed in two steps. The first step is to construct pT7H6FXΔTNFRSF13B by ligation of the Bgl II and Kpn I restricted DNA fragment FXΔTNFRSF13B amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bgliiFXΔTNFRSF13B (SEQ ID NO: 57) and ΔTNFRSF13Bkpn: (SEQ ID NO: 58)) into a BamH I and Kpn I cut vector, pT7H6-bktripB using standard

procedures. The second step is to construct the final expression vector pT7H6FXTNFRSF13B6tripB by ligation of the Kpn I restricted DNA fragment  $\Delta$ TNFRSF13B6 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers kpn $\Delta$ TNFSF13B (SEQ ID NO: 59) and  $\Delta$ TNFSF13B6kpn (SEQ ID NO: 60)) into a Kpn I cut pT7H6FX $\Delta$ TNFSF13B vector, using standard procedures. The resulting nucleotide sequence of FXTNFRSF13B6, is given as SEQ ID NO:61.

#### Cloning of TNFRSF13B10

The expression vector pT7H6FXTNFRS13B10tripB, is constructed in two steps. First step is to construct pT7H6FX $\Delta$ TNFRSF13B by ligation of the Bgl II and Kpn I restricted DNA fragment FX $\Delta$ TNFRSF13B amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bgliiFX $\Delta$ TNFRSF13B (SEQ ID NO: 57) and  $\Delta$ TNFRSF13Bkpn (SEQ ID NO: 58)) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. The second step is to construct the final expression vector pT7H6FXTNFRSF13B10tripB by ligation of the Kpn I restricted DNA fragment  $\Delta$ TNFSF13B10 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers kpn $\Delta$ TNFSF13B (SEQ ID NO: 59) and  $\Delta$ TNFSF13B10kpn (SEQ ID NO: 62)) into a Kpn I cut pT7H6FX $\Delta$ TNFRSF13B vector, using standard procedures. The resulting nucleotide sequence of FXTNFRSF13B10, is given as SEQ ID NO: 63.

#### Cloning of TNFRSF13B20

The expression vector pT7H6FXTNFRS13B20tripB, is constructed in two steps. First step is to construct pT7H6FX $\Delta$ TNFRSF13B by ligation of the Bgl II and Kpn I restricted DNA fragment FX $\Delta$ TNFSF13B amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bgliiFX $\Delta$ TNFRSF13B (SEQ ID NO: 57) and  $\Delta$ TNFRSF13Bkpn (SEQ ID NO: 58)) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. The second step is to construct the final expression vector pT7H6FXTNFRSF13B20tripB by ligation of the Kpn I restricted DNA fragment

$\Delta$ TNFSF13B20 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers kpn $\Delta$ TNFRSF13B (SEQ ID NO: 59) and  $\Delta$ TNFRSF13B20kpn (SEQ ID NO: 64)) into a Kpn I cut pT7H6FX $\Delta$ TNFRSF13B vector, using standard procedures. The resulting nucleotide sequence of FXTNFRSF13B20, is given as SEQ ID NO:65.

#### Cloning of TNFRSF13B30

The expression vector pT7H6FXTNFRSF13B30tripB, is constructed in two steps. First step is to construct pT7H6FX $\Delta$ TNFRSF13B by ligation of the Bgl II and Kpn I restricted DNA fragment FX $\Delta$ TNFRSF13B amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bglII FX $\Delta$ TNFRSF13B (SEQ ID NO: 57) and  $\Delta$ TNFRSF13Bkpn (SEQ ID NO: 58)) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. The second step is to construct the final expression vector pT7H6FXTNFRSF13B30tripB by ligation of the Kpn I restricted DNA fragment  $\Delta$ TNFRSF13B30 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers kpn $\Delta$ TNFRSF13B (SEQ ID NO: 59) and  $\Delta$ TNFRSF13B30kpn (SEQ ID NO: 66)) into a Kpn I cut pT7H6FX $\Delta$ TNFRSF13B vector, using standard procedures. The resulting nucleotide sequence of FXTNFRSF13B30, is given as SEQ ID NO:67.

#### Cloning of TNFRSF13B61

The expression vector pT7H6FXTNFRSF13B61tripB, is constructed in two steps. First step is to construct pT7H6FX $\Delta$ TNFRSF13B by ligation of the Bgl II and Kpn I restricted DNA fragment FX $\Delta$ TNFRSF13B amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers bglII FX $\Delta$ TNFRSF13B (SEQ ID NO: 57) and  $\Delta$ TNFRSF13Bkpn (SEQ ID NO: 58)) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. The second step is to construct the final expression vector pT7H6FXTNFRSF13B61tripB by ligation of the Kpn I restricted DNA fragment

$\Delta$ TNFRSF13B61 amplified from cDNA, isolated from a mixture of human bone marrow and human leukocyte (Clontech Laboratories, Inc cat # 7181-1 and 7182-1) (with the oligonucleotide primers kpn $\Delta$ TNFRSF13B (SEQ ID NO: 59) and  $\Delta$ TNFRSF13B61kpn (SEQ ID NO: 68)) into a Kpn I cut pT7H6FX $\Delta$ TNFRSF13B vector, using standard procedures. The resulting nucleotide sequence of FXTNFRSF13B61, is given as SEQ ID NO:69.

### **Example 5**

#### **Design and construction of a trimeric TWEAK binder using TNFRSF Fn14 receptor fragments**

The TWEAK receptor TNFRSF Fn14 is used for the construction of a trimeric binder against the trimeric cytokine TWEAK, and based on the trimerising domain TripB derived from tetranectin. The design and cloning of the binder is outlined in the following:

The expression vector pT7H6FXFn14tripB, is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXFn14 amplified from cDNA, isolated from human heart (Clontech Laboratories, Inc cat # 7121-1) (with the oligonucleotide primers bamFn-rev (SEQ ID NO: 70) and FNkpn-fo (SEQ ID NO:71) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXFn14, is given as SEQ ID NO:72.

### **Example 5**

#### **Design and construction of a trimeric BAFF binder using TNFRSF13C receptor fragments**

The BAFF receptor TNFRSF13B is used for the construction of a trimeric binder against the corresponding trimeric cytokine BAFF, and based on the trimerising domain TripB derived from tetranectin. The design and cloning of the binder is outlined in the following:

#### Cloning of trimeric TNFRSF13C fragment

The expression vector pT7H6FXTNFSF13CtripB , is constructed by ligation of the BamH I and Kpn I restricted DNA fragment FXTNFRSF13C amplified from cDNA, isolated from human Lymph Node(Clontech Laboratories, Inc cat # 7164-1) (with the oligonucleotide primers bam13C-rev (SEQ ID NO: 73) and 13Ckpn-fo (SEQ ID NO:74) into a BamH I and Kpn I cut vector, PT7H6-bktripB using standard procedures. Outlines of the resulting the nucleotide sequence of FXTNFRSF13C, is given as SEQ ID NO:75.

## **Claims**

- 1. A trimeric polypeptide comprising three monomers, each of said monomers comprising a specific binding member capable of binding a trimeric cytokine, and each of said monomers comprising a trimerising domain.**
- 2. A trimeric polypeptide according to claim 1, wherein the trimeric cytokine is a member of the Tumor necrosis factor ligand superfamily.**
- 3. A trimeric polypeptide according to claim 2, wherein the member of the Tumor necrosis factor ligand superfamily is selected from the group consisting of LTA; TNF; LTB; TNFSF4; TNFSF5; TNFSF6; TNFSF7; TNFSF8; TNFSF9; TNFSF10; TNFSF11; TNFSF12; TNFSF13; TNFSF13B; TNFSF14; TNFSF15; and TNFSF18.**
- 4. A trimeric polypeptide according to claim 2, wherein the specific binding member is a polypeptide derived from a member of the Tumor necrosis factor receptor superfamily.**
- 5. A trimeric polypeptide according to claim 2, wherein the member of the Tumor necrosis factor receptor superfamily is selected from the group consisting of TNFRSF1A, TNFRSF1B, LTBR, TNFRSF4, TNFRSF5, TNFRSF6, TNFRSF6B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF10A, TNFRSF10B, TNFRSF10C, TNFRSF10D, TNFRSF11A, TNFRSF11B, TNFRSF12, TNFRSF12L, TNFRSF13B, TNFRSF13C, TNFRSF14, TNFRSF Fn14, NGFR, TNFRSF17, TNFRSF18, TNFRSF19, TNFRSF19L, TNFRSF21, TNFRSF22, and TNFRSF23.**
- 6. A trimeric polypeptide according to claim 1, wherein the specific binding member is derived from TNFRSF1A (p55 TNF receptor).**
- 7. A trimeric polypeptide according to claim 1, wherein the specific binding member is derived from TNFRSF1B (p75 TNF receptor).**

8. A trimeric polypeptide according to claim 7, wherein the specific binding member is a polypeptide comprising an amino acid sequence selected from the group consisting of TNFRSF1B 1-235 (SEQ ID NO:76), TNFRSF1B 1-185 (SEQ ID NO:77), TNFRSF1B 1-163 (SEQ ID NO:78) and TNFRSF1B 1-142 (SEQ ID NO:79).

9. A trimeric polypeptide according to claim 7, wherein the specific binding member is a polypeptide comprising an amino acid sequence encoded by a DNA sequence selected from the group consisting of TNFRSF1B D1D2 (SEQ ID NO:13), TNFRSF1B D1D2, 1/6 (SEQ ID NO:15), TNFRSF1B D1D2 1/4 (SEQ ID NO:17), TNFRSF1B D1D2, 1/3 (SEQ ID NO:19), TNFRSF1B D1D2, 1/2 (SEQ ID NO:21), TNFRSF1B D1D4 (SEQ ID NO:23), TNFRSF1B D2 (SEQ ID NO:25), TNFRSF1B D2, 1/6 (SEQ ID NO:26), TNFRSF1B D2, 1/4 (SEQ ID NO:27), TNFRSF1B D2, 1/3 (SEQ ID NO:28), TNFRSF1B D2, 1/2 (SEQ ID NO:29) and TNFRSF1B D2D4 (SEQ ID NO:30).

10. A trimeric polypeptide according to claim 1, wherein the specific binding member is an antibody or antibody fragment.

11. A trimeric polypeptide according to claim 10, wherein the antibody or antibody fragment binds to human TNF (SEQ ID NO:80) in at least one region selected from the regions consisting of amino acid residues 1-18, 1-20, 1-26, 1-30, 12-22, 22-31, 22-40, 36-45, 49-97, 49-98, 56-79, 58-65, 69-97, 70-87, 76-90, 96-105, 105-128, 108-128, 110-127, 115-125, 117-128, 132-157, 135-155, 136-153, 138-149, 141-153 and 146-157.

12. A trimeric polypeptide according to claim 11, wherein the antibody or antibody fragment is selected from MAb 1 (ECACC 89080301), MAb 21 (ECACC 90012432), MAb 25 (ECACC 89121401), MAb 32 (ECACC 89080302), MAb 37 (ECACC 89080303), MAb 42 (ECACC 89080304), MAb 47 (ECACC 89121402), MAb 53 (ECACC 90012433) and MAb 54 (ECACC 89083103) or a fragment thereof.

13. A trimeric polypeptide according to claim 10, wherein the antibody is D2E7 or a fragment thereof.

**14. A trimeric polypeptide according to claim 9, wherein the antibody is CDP 870 or a fragment thereof.**

**15. A trimeric polypeptide according to claim 1, wherein the specific binding member is a protein having the scaffold structure of C-type lectin-like domains (CTLD).**

**16. A trimeric polypeptide according to claim 1, wherein the trimerising domain is derived from tetranectin.**

**17. A trimeric polypeptide according to claim 16, wherein the trimerising domain derived from tetranectin comprises a sequence having at least 68% amino acid sequence identity with the sequence of SEQ ID NO:81.**

**18. A trimeric polypeptide according to claim 17, wherein the amino acid sequence identity is at least 75%, such as at least 87% including at least 92%.**

**19. A trimeric polypeptide according to claim 16, wherein the trimerising domain derived from tetranectin comprises the amino acid sequence SEQ ID NO:81.**

**20. A trimeric polypeptide according to claim 1, further comprising a linker between the specific binding member and the trimerising domain.**

**21. A pharmaceutical composition comprising the trimeric polypeptide according to any of claims 1-20.**

**22. A method of treating a subject having a pathology mediated by tumor necrosis factor comprising administering to said subject an effective amount of the trimeric polypeptide according to any of claims 1-20 or the composition according to claim 21.**

**23. A method according to claim 22 wherein the pathology mediated by tumor necrosis factor is selected from the group consisting of rheumatoid arthritis, psoriasis and Crohn's disease.**



24. A method for the preparation of a trimeric polypeptide comprising three monomers, each of said monomers comprising a specific binding member capable of binding a trimeric polypeptide cytokine, and each of said monomers comprising a trimerising domain, said method comprising the steps of (i) culturing a host transformed with a vector encoding said trimeric polypeptide under such conditions that said trimeric polypeptide is expressed; and (ii) isolating said trimeric polypeptide.

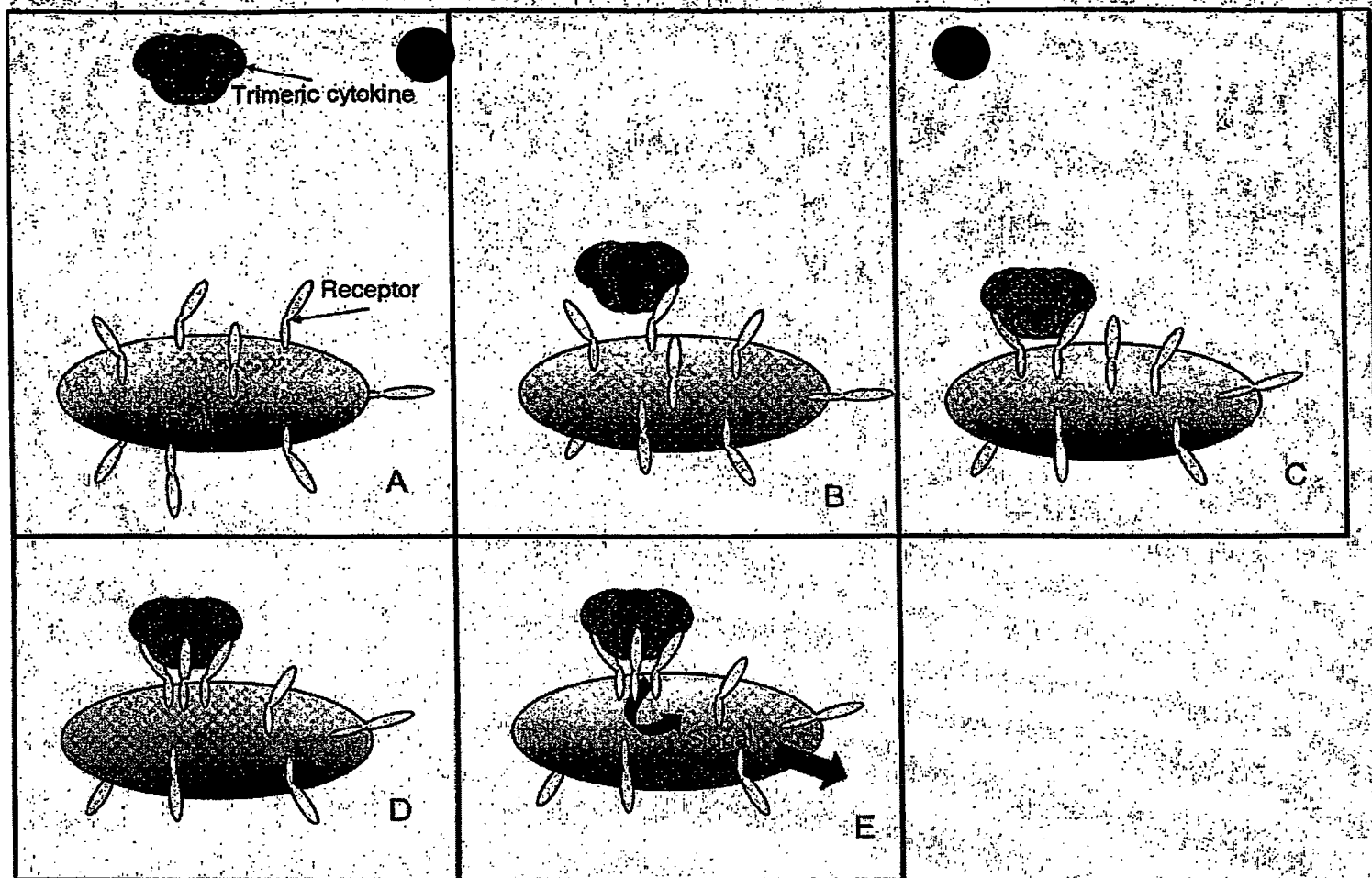
25. A method according to claim 24, wherein said specific binding member comprises an amino acid sequence selected from the group consisting of TNFRSF1B 1-235 (SEQ ID NO:76), TNFRSF1B 1-185 (SEQ ID NO:77), TNFRSF1B 1-163 (SEQ ID NO:78) and TNFRSF1B 1-142 (SEQ ID NO:79).

26. A method according to claim 24, wherein the specific binding member is a polypeptide comprising an amino acid sequence encoded by a DNA sequence selected from the group consisting of TNFRSF1B D1D2 (SEQ ID NO:13), TNFRSF1B D1D2, 1/6 (SEQ ID NO:15), TNFRSF1B D1D2 1/4 (SEQ ID NO:17), TNFRSF1B D1D2, 1/3 (SEQ ID NO:19), TNFRSF1B D1D2, 1/2 (SEQ ID NO:21), TNFRSF1B D1D4 (SEQ ID NO:23), TNFRSF1B D2 (SEQ ID NO:25), TNFRSF1B D2, 1/6 (SEQ ID NO:26), TNFRSF1B D2, 1/4 (SEQ ID NO:27), TNFRSF1B D2, 1/3 (SEQ ID NO:28), TNFRSF1B D2, 1/2 (SEQ ID NO:29) and TNFRSF1B D2D4 (SEQ ID NO:30).

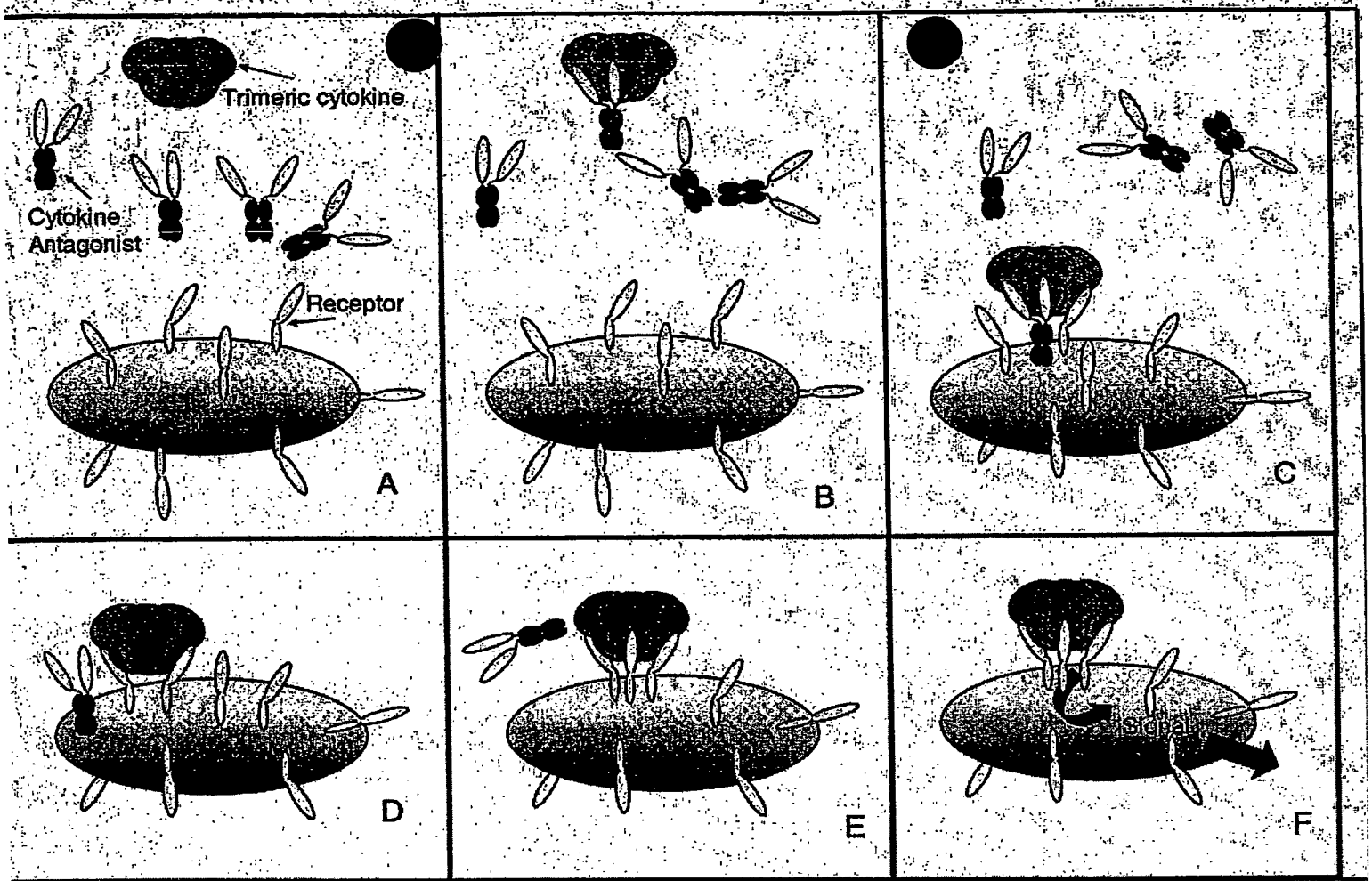
27. Use of a trimeric polypeptide according to any of claims 1-20.

28. Use of a trimeric polypeptide according to any of claims 1-20 for the preparation of a pharmaceutical composition.

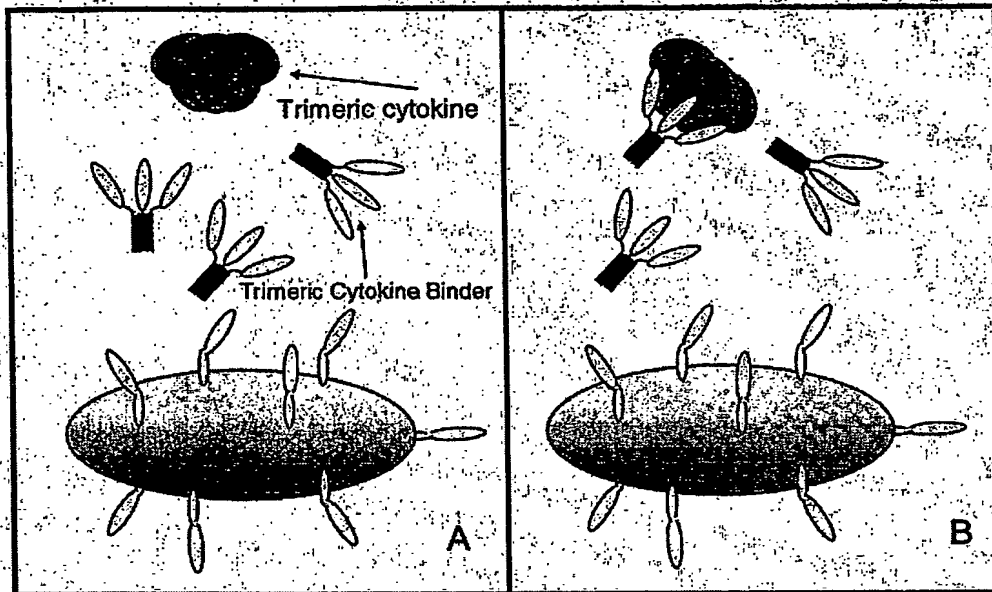
29. An assay method for detecting a trimeric cytokine in a sample comprising (i) contacting said sample with a trimeric polypeptide according to claim 1, and (ii) detecting the binding of the trimeric polypeptide to the trimeric cytokine.



**FIGURE 1**



**FIGURE 2**



**FIGURE 3**

SEQUENCE LISTING

Modtaget

29. OKT. 2002

PVS

<110> Borean Pharma A/S

<120> Trimeric cytokine binder

<130> 3005

<160> 81

<170> PatentIn version 3.1

<210> 1

<211> 33

<212> DNA

<213> Homo sapiens

<400> 1

ctgccatgcg gccgccttca gggagaccgt ctg

33

<210> 2

<211> 58

<212> DNA

<213> Homo sapiens

<400> 2

gtgaccaggc ccagccggcc atgtaagtag ctaaggtacc gagccaccaa cccagaag

58

<210> 3

<211> 197

<212> DNA

<213> Homo sapiens

<400> 3

ggcccagccg gccatgtaag tagctaaggt accgagccac caaccagaa gccaagaag

60

attgtaaatg ccaagaaaga tgttgtgaac acaaagatgt ttgaggagct caagagccgt

120

ctggacaccc tggcccagga ggtggccctg ctgaaggagc agcaggccct gcagacggtc

180

tccctgaagg cggccgc

197

<210> 4

<211> 62

<212> DNA

<213> Homo sapiens

<400> 4

gtgaccaggc ccagccggcc atgtaagtag ctaaggtacc attgtaaatg ccaagaaaga

60

tg

62

<210> 5

<211> 170

<212> DNA

<213> Homo sapiens

<400> 5

ggcccagccg gccatgtaag tagctaaggt accattgtaa atgccaagaa agatgttgtg

60

aacacaaaga tgtttgagga gctcaagagc cgtctggaca ccctggccca ggaggtggcc 120  
ctgctgaagg agcagcaggc cctgcagacg gtctccctga aggcggccgc 170

<210> 6  
<211> 32  
<212> DNA  
<213> Homo sapiens

<400> 6  
ctgccataag cttacttcag ggagaccgtc tg 32

<210> 7  
<211> 51  
<212> DNA  
<213> Homo sapiens

<400> 7  
gtgaccagga tccatgtaag tagctaaggt accgagccac caaccagaa g 51

<210> 8  
<211> 191  
<212> DNA  
<213> Homo sapiens

<400> 8  
ggatccatgt aagtagctaa ctgcaggtga gccaccaacc cagaagccca agaagattgt 60  
aaatgccaaag aaagatgttg tgaacacaaa gatgtttgag gagctcaaga gccgtctgga 120  
caccctggcc caggaggtgg ccctgctgaa ggagcagcag gccctgcaga cggctctccct 180  
gaagtaagct t 191

<210> 9  
<211> 55  
<212> DNA  
<213> Homo sapiens

<400> 9  
gtgaccagga tccatgtaag tagctaaggt accattgtaa atgccaagaa agatg 55

<210> 10  
<211> 162  
<212> DNA  
<213> Homo sapiens

<400> 10  
ggatccatgt aagtagctaa ggtaccattg taaatgccaa gaaagatgtt gtgaacacaa 60  
agatgtttga ggagctcaag agccgtcttg acaccctggc ccaggaggtg gccctgctga 120  
aggagcagca ggccctgcag acggtctccc tgaagtaagc tt 162

<210> 11  
<211> 44  
<212> DNA  
<213> Homo sapiens

<400> 11  
gtgaccaggc ccagccggcc atggccggga gcacatgccg gctc

44

<210> 12  
<211> 34  
<212> DNA  
<213> Homo sapiens

<400> 12  
cgtgctgggt accggtgcag atgcggttct gtcc

34

<210> 13  
<211> 274  
<212> DNA  
<213> Homo sapiens

<400> 13  
ggccagccg gccatggccg ggagcacatg ccggctcaga gaatactatg accagacagc 60  
tcagatgtgc tgcagcaagt gctcgccggg ccaacatgca aaagtcttct gtaccaagac 120  
ctcggacacc gtgtgtgact cctgtgagga cagcacatac acccagctct ggaactgggt 180  
tcccagagtgc ttgagctgtg gctcccgtg tagctctgac caggtggaaa ctcaagcctg 240  
cactcgggaa cagaaccgca tctgcaccgg tacc 274

<210> 14  
<211> 39  
<212> DNA  
<213> Homo sapiens

<400> 14  
cgtgctgggt accgggccta gcggtgcaga tgcggttct

39

<210> 15  
<211> 283  
<212> DNA  
<213> Homo sapiens

<400> 15  
ggcccagccg gccatggccg ggagcacatg ccggctcaga gaatactatg accagacagc 60  
tcagatgtgc tgcagcaagt gctcgccggg ccaacatgca aaagtcttct gtaccaagac 120  
ctcggacacc gtgtgtgact cctgtgagga cagcacatac acccagctct ggaactgggt 180  
tcccagagtgc ttgagctgtg gctcccgtg tagctctgac caggtggaaa ctcaagcctg 240  
cactcgggaa cagaaccgca tctgcaccgc taggcccgggt acc 283

<210> 16  
<211> 31  
<212> DNA  
<213> Homo sapiens

<400> 16  
cgtgctgggt acccgccgag taccagccgg g

31

<210> 17  
 <211> 298  
 <212> DNA  
 <213> Homo sapiens

<400> 17  
 ggcccagccg gccatggccg ggagcacatg ccggctcaga gaatactatg accagacagc 60  
 tcagatgtgc tgcagcaagt gctcgccggg ccaacatgca aaagtcttct gtaccaagac 120  
 ctcggacacc gtgtgtgact cctgtgagga cagcacatac acccagctct ggaactgggt 180  
 tcccagagtgc ttgagctgtg gctcccgtg tagctctgac caggtggaaa ctcaagcctg 240  
 cactcgggaa cagaaccgca tctgcacctg caggcccggc tgggtactgcg cgggtacc 298

<210> 18  
 <211> 29  
 <212> DNA  
 <213> Homo sapiens

<400> 18  
 cgtgctgggt accctcctgc ttgctcagc 29

<210> 19  
 <211> 316  
 <212> DNA  
 <213> Homo sapiens

<400> 19  
 ggcccagccg gccatggccg ggagcacatg ccggctcaga gaatactatg accagacagc 60  
 tcagatgtgc tgcagcaagt gctcgccggg ccaacatgca aaagtcttct gtaccaagac 120  
 ctcggacacc gtgtgtgact cctgtgagga cagcacatac acccagctct ggaactgggt 180  
 tcccagagtgc ttgagctgtg gctcccgtg tagctctgac caggtggaaa ctcaagcctg 240  
 cactcgggaa cagaaccgca tctgcacctg caggcccggc tgggtactgcg cgctgagcaa 300  
 gcaggagggg ggtacc 316

<210> 20  
 <211> 44  
 <212> DNA  
 <213> Homo sapiens

<400> 20  
 cgtgctgggt acctgcagcc agccgagccc cctcctgctt gctc 44

<210> 21  
 <211> 331  
 <212> DNA  
 <213> Homo sapiens

<400> 21  
 ggcccagccg gccatggccg ggagcacatg ccggctcaga gaatactatg accagacagc 60



tcagatgtgc tgcagcaagt gctcgccggg ccaacatgca aaagtcttct gtaccaagac	120
ctcggacacc gtgtgtgact cctgtgagga cagcacatac acccagctct ggaactgggt	180
tcccagagtgc ttgagctgtg gctcccgtg tagctctgac caggtggaaa ctcaagcctg	240
cactcgggaa cagaaccgca tctgcacctg caggcccggc tggtagtgcg cgctgagcaa	300
gcaggagggg gctcggctgg ctgcaggtac c	331

<210> 22  
 <211> 31  
 <212> DNA  
 <213> Homo sapiens

<400> 22	
cgtagctgggt accggacgtg gacgtgcaga c	31

<210> 23  
 <211> 529  
 <212> DNA  
 <213> Homo sapiens

<400> 23	
ggcccagccg gccatggccg ggagcacatg ccggctcaga gaatactatg accagacagc	60
tcagatgtgc tgcagcaagt gctcgccggg ccaacatgca aaagtcttct gtaccaagac	120
ctcggacacc gtgtgtgact cctgtgagga cagcacatac acccagctct ggaactgggt	180
tcccagagtgc ttgagctgtg gctcccgtg tagctctgac caggtggaaa ctcaagcctg	240
cactcgggaa cagaaccgca tctgcacctg caggcccggc tggtagtgcg cgctgagcaa	300
gcaggagggg tgccggctgt gcgcgccgt gcgcaagtgc cgccgggct tcggcgtggc	360
cagaccagga actgaaacat cagacgtggt gtgcaagccc tgtgccccgg ggacgttctc	420
caacacgact tcatccacgg atatttgcag gccccaccag atctgtaacg tggtagccat	480
ccctgggaat gcaagcaggg atgcagtctg cacgtccacg tccggtacc	529

<210> 24  
 <211> 44  
 <212> DNA  
 <213> Homo sapiens

<400> 24	
gtgaccaggc ccagccggcc atggccgact cctgtgagga cagc	44

<210> 25  
 <211> 157  
 <212> DNA  
 <213> Homo sapiens

<400> 25	
ggcccagccg gccatggccg actcctgtga ggacagcaca tacaccagc tctggaactg	60
ggttcccagag tgcttgagct gtggctcccg ctgtagctct gaccaggtgg aaactcaagc	120

ctgcactcgg gaacagaacc gcatctgcac cggtacc

157

<210> 26  
<211> 166  
<212> DNA  
<213> Homo sapiens

<400> 26  
ggcccagccg gccatggccg actcctgtga ggacagcaca tacaccagc tctggaactg 60  
ggttcccag tgcttgagct gtggctcccg ctgtagctct gaccaggtgg aaactcaagc 120  
ctgcactcgg gaacagaacc gcatctgcac cgctaggccc ggtacc 166

<210> 27  
<211> 182  
<212> DNA  
<213> Homo sapiens

<400> 27  
ggcccagccg gccatggcct gactcctgtg aggacagcac atacaccag ctctggaact 60  
gggttcccga gtgcttgagc tgtggctccc gctgtagctc tgaccaggtg gaaactcaag 120  
cctgcactcg ggaacagaac cgcacttgca cctgcaggcc cggctggtac tgcgcgggta 180  
cc 182

<210> 28  
<211> 199  
<212> DNA  
<213> Homo sapiens

<400> 28  
ggcccagccg gccatggccg actcctgtga ggacagcaca tacaccagc tctggaactg 60  
ggttcccag tgcttgagct gtggctcccg ctgtagctct gaccaggtgg aaactcaagc 120  
ctgcactcgg gaacagaacc gcatctgcac ctgcaggccc ggctggtact gcgcgctgag 180  
caagcaggag gggggtacc 199

<210> 29  
<211> 214  
<212> DNA  
<213> Homo sapiens

<400> 29  
ggcccagccg gccatggccg actcctgtga ggacagcaca tacaccagc tctggaactg 60  
ggttcccag tgcttgagct gtggctcccg ctgtagctct gaccaggtgg aaactcaagc 120  
ctgcactcgg gaacagaacc gcatctgcac ctgcaggccc ggctggtact gcgcgctgag 180  
caagcaggag ggggctcggc tggctgcagg tacc 214

<210> 30  
<211> 412  
<212> DNA

<213> Homo sapiens

<400> 30  
ggcccagccg gccatggccg actcctgtga ggacagcaca tacacccagc tctggaactg 60  
ggttcccagag tgcttgagct gtggctcccg ctgtagctct gaccagggtg aaactcaagc 120  
ctgcactcgg gaacagaacc gcatctgcac ctgcaggccc ggctgggtact gcgcgctgag 180  
caagcaggag ggggtgccggc tgtgcgcgcc gctgcgcaag tgccgcccgg gcttcggcgt 240  
ggccagacca ggaactgaaa catcagacgt ggtgtgcaag ccctgtgccc cggggacgtt 300  
ctccaacacg acttcatcca cggatatttg caggccccac cagatctgta acgtgggtggc 360  
catccctggg aatgcaagca gggatgcagt ctgcacgtcc acgtccggta cc 412

<210> 31  
<211> 41  
<212> DNA  
<213> Homo sapiens

<400> 31  
gccagggatc catcgagggt agggggagca catgccggct c 41

<210> 32  
<211> 278  
<212> DNA  
<213> Homo sapiens

<400> 32  
gccagggatc catcgagggt agggggagca catgccggct cagagaatac tatgaccaga 60  
cagctcagat gtgctgcagc aagtgtctgc cgggccaaca tgcaaaagtc ttctgtacca 120  
agacctcgga caccgtgtgt gactcctgtg aggacagcac atacacccag ctctggaact 180  
gggttcccga gtgcttgagc tgtggctccc gctgtagctc tgaccagggtg gaaactcaag 240  
cctgcactcg ggaacagaac cgcactctgca ccggtacc 278

<210> 33  
<211> 287  
<212> DNA  
<213> Homo sapiens

<400> 33  
gccagggatc catcgagggt agggggagca catgccggct cagagaatac tatgaccaga 60  
cagctcagat gtgctgcagc aagtgtctgc cgggccaaca tgcaaaagtc ttctgtacca 120  
agacctcgga caccgtgtgt gactcctgtg aggacagcac atacacccag ctctggaact 180  
gggttcccga gtgcttgagc tgtggctccc gctgtagctc tgaccagggtg gaaactcaag 240  
cctgcactcg ggaacagaac cgcactctgca ccgctaggcc cgggtacc 287

<210> 34  
<211> 302  
<212> DNA

<213> Homo sapiens

<400> 34  
gccagggatc catcgagggt agggggagca catgccggct cagagaatac tatgaccaga 60  
cagctcagat gtgctgcagc aagtgtctgc cgggcccaaca tgcaaaagtc ttctgtacca 120  
agacctcgga caccgtgtgt gactcctgtg aggacagcac atacaccag ctctggaact 180  
gggttcccga gtgcttgagc tgtggctccc gctgtagctc tgaccaggtg gaaactcaag 240  
cctgcactcg ggaacagaac cgcactctgca cctgcaggcc cggctggtac tgcgcgggta 300  
cc 302

<210> 35  
<211> 320  
<212> DNA  
<213> Homo sapiens

<400> 35  
gccagggatc catcgagggt agggggagca catgccggct cagagaatac tatgaccaga 60  
cagctcagat gtgctgcagc aagtgtctgc cgggcccaaca tgcaaaagtc ttctgtacca 120  
agacctcgga caccgtgtgt gactcctgtg aggacagcac atacaccag ctctggaact 180  
gggttcccga gtgcttgagc tgtggctccc gctgtagctc tgaccaggtg gaaactcaag 240  
cctgcactcg ggaacagaac cgcactctgca cctgcaggcc cggctggtac tgcgcgctga 300  
gcaagcagga ggggggtacc 320

<210> 36  
<211> 335  
<212> DNA  
<213> Homo sapiens

<400> 36  
gccagggatc catcgagggt agggggagca catgccggct cagagaatac tatgaccaga 60  
cagctcagat gtgctgcagc aagtgtctgc cgggcccaaca tgcaaaagtc ttctgtacca 120  
agacctcgga caccgtgtgt gactcctgtg aggacagcac atacaccag ctctggaact 180  
gggttcccga gtgcttgagc tgtggctccc gctgtagctc tgaccaggtg gaaactcaag 240  
cctgcactcg ggaacagaac cgcactctgca cctgcaggcc cggctggtac tgcgcgctga 300  
gcaagcagga gggggctcgg ctggctgcag gtacc 335

<210> 37  
<211> 533  
<212> DNA  
<213> Homo sapiens

<400> 37  
gccagggatc catcgagggt agggggagca catgccggct cagagaatac tatgaccaga 60  
cagctcagat gtgctgcagc aagtgtctgc cgggcccaaca tgcaaaagtc ttctgtacca 120

agacctcgga caccgtgtgt gactcctgtg aggacagcac atacacccag ctctggaact	180
gggttcccga gtgcttgagc tgtggctccc gctgtagctc tgaccagggtg gaaactcaag	240
cctgcactcg ggaacagaac cgcattctgca cctgcaggcc cggctggtac tgcgcgctga	300
gcaagcagga ggggtgccgg ctgtgcgcgc cgctgcgcaa gtgccgcccg ggcttcggcg	360
tggccagacc aggaactgaa acatcagacg tgggtgtgcaa gccctgtgcc ccggggacgt	420
tctccaacac gacttcattc acggatattt gcaggcccca ccagatctgt aacgtggtgg	480
ccatccctgg gaatgcaagc agggatgcag tctgcacgtc cacgtccggt acc	533

<210> 38  
 <211> 44  
 <212> DNA  
 <213> Homo sapiens

<400> 38	
gccagggatc catcgagggt aggggcgact cctgtgagga cagc	44

<210> 39  
 <211> 159  
 <212> DNA  
 <213> Homo sapiens

<400> 39	
ggatccatcg agggtagggg cgactcctgt gaggacagca catacaccca gctctggaac	60
tgggttcccg agtgcttgag ctgtggctcc cgctgtagct ctgaccagggt ggaaactcaa	120
gcctgcactc gggaacagaa ccgcattctgc accggtacc	159

<210> 40  
 <211> 168  
 <212> DNA  
 <213> Homo sapiens

<400> 40	
ggatccatcg agggtagggg cgactcctgt gaggacagca catacaccca gctctggaac	60
tgggttcccg agtgcttgag ctgtggctcc cgctgtagct ctgaccagggt ggaaactcaa	120
gcctgcactc gggaacagaa ccgcattctgc accgctaggc ccggtacc	168

<210> 41  
 <211> 183  
 <212> DNA  
 <213> Homo sapiens

<400> 41	
ggatccatcg agggtagggg cgactcctgt gaggacagca catacaccca gctctggaac	60
tgggttcccg agtgcttgag ctgtggctcc cgctgtagct ctgaccagggt ggaaactcaa	120
gcctgcactc gggaacagaa ccgcattctgc acctgcaggc ccggctggta ctgcgcgggt	180
acc	183

<210> 42  
 <211> 201  
 <212> DNA  
 <213> Homo sapiens

<400> 42  
 ggatccatcg agggtagggg cgactcctgt gaggacagca catacaccca gctctggaac 60  
 tgggttcccg agtgcttgag ctgtggctcc cgctgtagct ctgaccaggt ggaaactcaa 120  
 gcctgcactc gggaacagaa ccgcatctgc acctgcaggc ccggctggta ctgcgcgctg 180  
 agcaagcagg aggggggtac c 201

<210> 43  
 <211> 216  
 <212> DNA  
 <213> Homo sapiens

<400> 43  
 ggatccatcg agggtagggg cgactcctgt gaggacagca catacaccca gctctggaac 60  
 tgggttcccg agtgcttgag ctgtggctcc cgctgtagct ctgaccaggt ggaaactcaa 120  
 gcctgcactc gggaacagaa ccgcatctgc acctgcaggc ccggctggta ctgcgcgctg 180  
 agcaagcagg aggggggtcg gctggctgca ggtacc 216

<210> 44  
 <211> 414  
 <212> DNA  
 <213> Homo sapiens

<400> 44  
 ggatccatcg agggtagggg cgactcctgt gaggacagca catacaccca gctctggaac 60  
 tgggttcccg agtgcttgag ctgtggctcc cgctgtagct ctgaccaggt ggaaactcaa 120  
 gcctgcactc gggaacagaa ccgcatctgc acctgcaggc ccggctggta ctgcgcgctg 180  
 agcaagcagg aggggtgccg gctgtgcgcg ccgctgcgca agtgccgcc gggcttcggc 240  
 gtggccagac caggaactga aacatcagac gtgggtgtgca agccctgtgc cccggggacg 300  
 ttctccaaca cgacttcac caccgatatt tgcaggcccc accagatctg taacgtggtg 360  
 gccatccctg ggaatgcaag cagggatgca gtctgcacgt ccacgtccgg tacc 414

<210> 45  
 <211> 41  
 <212> DNA  
 <213> Homo sapiens

<400> 45  
 gccagagatc tatcgagggt agggaggtgc agctggtgga g 41

<210> 46  
 <211> 59  
 <212> DNA

<213> Homo sapiens

<400> 46  
ctggtcggta ccgcatccgc cgccaccact cgagacgggtg accagagtac cttggcccc 59

<210> 47  
<211> 62  
<212> DNA  
<213> Homo sapiens

<400> 47  
gcaggcggat ccgggggagg aggtagtggc ggtggtggat cagacatcca gatgaccag 60  
tc 62

<210> 48  
<211> 33  
<212> DNA  
<213> Homo sapiens

<400> 48  
ccgagcggta cctttgattt ccaccttggc ccc 33

<210> 49  
<211> 754  
<212> DNA  
<213> Homo sapiens

<400> 49  
agatctatcg agggtaggga ggtgcagctg gtggagtctg ggggaggctt ggtacagccc 60  
ggcagggtccc tgagactctc ctgtgcggcc tctggattca cctttgatga ttatgccatg 120  
cactgggtcc ggcaagctcc aggggaagggc ctggaatggg tctcagctat cacttggaaat 180  
agtggtcaca tagactatgc ggactctgtg gagggccgat tcaccatctc cagagacaac 240  
gccaaagaact ccctgtatct gcaaatgaac agtctgagag ctgaggatac ggccgtatat 300  
tactgtgcga aagtctcgta ccttagcacc gcgtcctccc ttgactattg gggccaaggc 360  
accctgggtca ccgtctcgag tgggtggcggc gggatccggg ggaggaggta gtggcgggtg 420  
tggatcagac atccagatga cccagtctcc atcctccctg tctgcatctg taggggacag 480  
agtcaccatc acttgtcggg caagtcaggg catcagaaat tacttagcct ggtatcagca 540  
aaaaccaggg aaagccccta agctcctgat ctatgctgca tccactttgc aatcaggggt 600  
cccatctcgg ttcagtggca gtggatctgg gacagatttc actctcacca tcagcagcct 660  
acagcctgaa gatgttgcaa cttattactg tcaaaggat aaccgtgcac cgtatacttt 720  
tggccagggg accaagggtg aaatcaaagg tacc 754

<210> 50  
<211> 48  
<212> DNA  
<213> Homo sapiens

<400> 50  
ccgagcggta ccagatccac cgcccccttt gatttccacc ttgggtccc

48

<210> 51  
<211> 769  
<212> DNA  
<213> Homo sapiens

<400> 51  
agatctatcg agggtaggga ggtgcagctg gtggagtctg ggggaggctt ggtacagccc 60  
ggcagggtccc tgagactctc ctgtgcggcc tctggattca cctttgatga ttatgccatg 120  
cactgggtcc ggcaagctcc agggaagggc ctggaatggg tctcagctat cacttggaat 180  
agtggtcaca tagactatgc ggactctgtg gagggccgat tcaccatctc cagagacaac 240  
gccaaagaact ccctgtatct gcaaatgaac agtctgagag ctgaggatac ggccgtatat 300  
tactgtgcga aagtctcgta ccttagcacc gcgtcctccc ttgactattg gggccaagggt 360  
accctgggtca ccgtctcgag tgggtggcggc gggatccggg ggaggaggta gtggcgggtg 420  
tggatcagac atccagatga ccagctctcc atcctccctg tctgcatctg taggggacag 480  
agtcaccatc acttgtcggg caagtcaggg catcagaaat tacttagcct ggtatcagca 540  
aaaaccaggg aaagccccta agctcctgat ctatgctgca tccactttgc aatcaggggt 600  
cccattctcg tttagtgga gtggatctgg gacagatttc actctcacca tcagcagcct 660  
acagcctgaa gatgttgcaa cttattactg tcaaaggat aaccgtgcac cgtatacttt 720  
tggccagggg accaagggtg aaatcaaagg gggcgggtgga tctggtacc 769

<210> 52  
<211> 29  
<212> DNA  
<213> Homo sapiens

<400> 52  
gccagtgatc agagggtgcag ctggtggag

29

<210> 53  
<211> 33  
<212> DNA  
<213> Homo sapiens

<400> 53  
cctcgaagct tatttgattt ccaccttggt ccc

33

<210> 54  
<211> 743  
<212> DNA  
<213> Homo sapiens

<400> 54  
tgatcagagg tgcagctggt ggagtctggg ggaggcttgg tacagcccgg cagggtccctg 60  
agactctcct gtgcggcctc tggattcacc tttgatgatt atgccatgca ctgggtccgg 120



caagctccag ggaagggcct ggaatgggtc tcagctatca cttggaatag tggtcacata	180
gactatgcgg actctgtgga gggccgattc accatctcca gagacaacgc caagaactcc	240
ctgtatctgc aaatgaacag tctgagagct gaggatacgg ccgtatatta ctgtgcgaaa	300
gtctcgtacc ttagcaccgc gtcctccctt gactattggg gccaaggtag cctgggtcacc	360
gtctcgagtg gtggcgggcg gatccggggg aggaggtagt ggcgggtggtg gatcagacat	420
ccagatgacc cagtctccat cctccctgtc tgcattctgta ggggacagag tcaccatcac	480
ttgtcgggca agtcagggca tcagaaatta cttagcctgg tatcagcaaa aaccagggaa	540
agcccctaag ctcctgatct atgtctgcat cactttgcaa tcaggggtcc catctcggtt	600
cagtggcagt ggatctggga cagatttcac tctcaccatc agcagcctac agcctgaaga	660
tgttgcaact tattactgtc aaaggataaa ccgtgcaccg tatacttttg gccaggggac	720
caaggtggaa atcaaataag ctt	743

<210> 55  
 <211> 44  
 <212> DNA  
 <213> Homo sapiens

<400> 55	
gccagtgatc aggaggtggc gggctctgagg tgcagctggt ggag	44

<210> 56  
 <211> 758  
 <212> DNA  
 <213> Homo sapiens

<400> 56	
tgatcaggag gtggcgggtc tgaggtgcag ctggtggagt ctgggggagg cttggtacag	60
cccggcagggt ccctgagact ctctgtgctg gcctctggat tcacctttga tgattatgcc	120
atgcactggg tccggcaagc tccagggaag ggcctggaat gggctctcagc tatcacttgg	180
aatagtgggtc acatagacta tgcggactct gtggagggcc gattcaccat ctccagagac	240
aacgccaaga actccctgta tctgcaaata aacagtctga gagctgagga tacggccgta	300
tattactgtg cgaaagtctc gtaccttagc accgcgtcct cccttgacta ttggggccaa	360
ggatccctgg tcaccgtctc gagtgggtggc ggcgggatcc gggggaggag gtagtggcgg	420
tggtggatca gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtagggga	480
cagagtcacc atcacttgtc gggcaagtca gggcatcaga aattacttag cctgggtatca	540
gcaaaaacca gggaaaagccc ctaagctcct gatctatgct gcatccactt tgcaatcagg	600
gggtcccatct cggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag	660
cctacagcct gaagatgttg caacttatta ctgtcaaagg tataaccgtg caccgtatc	720
ttttggccag gggaccaagg tggaaatcaa ataagctt	758

<210> 57  
 <211> 38  
 <212> DNA  
 <213> Homo sapiens

<400> 57  
 gccagagatc tatcgagggt aggatgagtg gcctgggc 38

<210> 58  
 <211> 18  
 <212> DNA  
 <213> Homo sapiens

<400> 58  
 catgcaggta cccagcag 18

<210> 59  
 <211> 18  
 <212> DNA  
 <213> Homo sapiens

<400> 59  
 ctgctgggta cctgcatg 18

<210> 60  
 <211> 32  
 <212> DNA  
 <213> Homo sapiens

<400> 60  
 cggcacggta ccgctcctga gcttggtctc ac 32

<210> 61  
 <211> 354  
 <212> DNA  
 <213> Homo sapiens

<400> 61  
 agatctatcg agggtaggat gagtggcctg ggccggagca ggcgaggtgg ccggagccgt 60  
 gtggaccagg aggagcgctt tccacagggc ctgtggacgg gggtaggtat gagatcctgc 120  
 cccgaagagc agtactggga tcctctgctg ggtacctgca tgtcctgcaa aaccatttgc 180  
 aaccatcaga gccagcgcac ctgtgcagcc ttctgcaggt cactcagctg ccgcaaggag 240  
 caaggcaagt tctatgacca tctcctgagg gactgcatca gctgtgcctc catctgtgga 300  
 cagcacccta agcaatgtgc atacttctgt gagaacaagc tcaggagcgg tacc 354

<210> 62  
 <211> 31  
 <212> DNA  
 <213> Homo sapiens

<400> 62  
 cggcacggta ccaagggtca ctgggctcct g 31

<210> 63  
 <211> 366  
 <212> DNA  
 <213> Homo sapiens

<400> 63  
 agatctatcg agggtaggat gaggggcctg ggccggagca ggcgaggtgg ccggagccgt 60  
 gtggaccagg aggagcgctt tccacagggc ctgtggacgg gggaggctat gagatcctgc 120  
 cccgaagagc agtactggga tcctctgctg ggtacctgca tgcctgcaa aaccatttgc 180  
 aaccatcaga gccagcgcac ctgtgcagcc ttctgcaggt cactcagctg ccgcaaggag 240  
 caaggcaagt tctatgacca tctcctgagg gactgcatca gctgtgcctc catctgtgga 300  
 cagcacccta agcaatgtgc atacttctgt gagaacaagc tcaggagccc agtgaacctt 360  
 ggtacc 366

<210> 64  
 <211> 31  
 <212> DNA  
 <213> Homo sapiens

<400> 64  
 cggcacggta cctccactcc gctgtctcct g 31

<210> 65  
 <211> 396  
 <212> DNA  
 <213> Homo sapiens

<400> 65  
 agatctatcg agggtaggat gaggggcctg ggccggagca ggcgaggtgg ccggagccgt 60  
 gtggaccagg aggagcgctt tccacagggc ctgtggacgg gggaggctat gagatcctgc 120  
 cccgaagagc agtactggga tcctctgctg ggtacctgca tgcctgcaa aaccatttgc 180  
 aaccatcaga gccagcgcac ctgtgcagcc ttctgcaggt cactcagctg ccgcaaggag 240  
 caaggcaagt tctatgacca tctcctgagg gactgcatca gctgtgcctc catctgtgga 300  
 cagcacccta agcaatgtgc atacttctgt gagaacaagc tcaggagccc agtgaacctt 360  
 ccaccagagc tcaggagaga gcggagtgga ggtacc 396

<210> 66  
 <211> 36  
 <212> DNA  
 <213> Homo sapiens

<400> 66  
 cggcacggta ccagggtca acagacttaa caaaag 36

<210> 67  
 <211> 426

<212> DNA  
<213> Homo sapiens

<400> 67  
 agatctatcg agggtaggat gagtggcctg ggccggagca ggcgaggtgg ccggagccgt 60  
 gtggaccagg aggagcgctt tccacagggc ctgtggacgg ggggtggctat gagatcctgc 120  
 cccgaagagc agtactggga tcctctgctg ggtacctgca tgtcctgcaa aaccatttgc 180  
 aaccatcaga gccagcgcac ctgtgcagcc ttctgcaggt cactcagctg ccgcaaggag 240  
 caaggcaagt tctatgacca tctcctgagg gactgcatca gctgtgcctc catctgtgga 300  
 cagcacccta agcaatgtgc atacttctgt gagaacaagc tcaggagccc agtgaacctt 360  
 ccaccagagc tcaggagaca gcggagtgga gaagttgaaa acaattcaga caactcggga 420  
 ggtacc 426

<210> 68  
 <211> 31  
 <212> DNA  
 <213> Homo sapiens

<400> 68  
 cggcacggta ccgctgtaga ccagggccac c 31

<210> 69  
 <211> 519  
 <212> DNA  
 <213> Homo sapiens

<400> 69  
 agatctatcg agggtaggat gagtggcctg ggccggagca ggcgaggtgg ccggagccgt 60  
 gtggaccagg aggagcgctt tccacagggc ctgtggacgg ggggtggctat gagatcctgc 120  
 cccgaagagc agtactggga tcctctgctg ggtacctgca tgtcctgcaa aaccatttgc 180  
 aaccatcaga gccagcgcac ctgtgcagcc ttctgcaggt cactcagctg ccgcaaggag 240  
 caaggcaagt tctatgacca tctcctgagg gactgcatca gctgtgcctc catctgtgga 300  
 cagcacccta agcaatgtgc atacttctgt gagaacaagc tcaggagccc agtgaacctt 360  
 ccaccagagc tcaggagaca gcggagtgga gaagttgaaa acaattcaga caactcggga 420  
 aggtaccaag gattggagca cagaggctca gaagcaagtc cagctctccc ggggctgaag 480  
 ctgagtgcag atcaggtggc cctggtctac agcgggtacc 519

<210> 70  
 <211> 41  
 <212> DNA  
 <213> Homo sapiens

<400> 70  
 ggccagggat ccatcgaggg taggggggag caagcgccag g 41

<210> 71  
 <211> 31  
 <212> DNA  
 <213> Homo sapiens

<400> 71  
 cggtgcggtgta ccgggccaaa gcagccggaa g 31

<210> 72  
 <211> 186  
 <212> DNA  
 <213> Homo sapiens

<400> 72  
 ggatccatcg agggtagggg ggagcaagcg ccaggcaccg cccctgctc ccgcggcagc 60  
 tcctggagcg cggacctgga caagtgcag gactgcgcgt cttgcagggc gcgaccgcac 120  
 agcgacttct gcctgggctg cgctgcagca cctcctgccc ccttccggct gctttggccc 180  
 ggtacc 186

<210> 73  
 <211> 41  
 <212> DNA  
 <213> Homo sapiens

<400> 73  
 ggccagggat ccatcgaggg taggatgagg cgagggcccc g 41

<210> 74  
 <211> 28  
 <212> DNA  
 <213> Homo sapiens

<400> 74  
 cggtgcggtgta ccgagcagcc cgggcagg 28

<210> 75  
 <211> 258  
 <212> DNA  
 <213> Homo sapiens

<400> 75  
 ggatccatcg agggtaggat gaggcgaggg ccccgagcc tgcggggcag ggacgcgcca 60  
 gccccacgc cctgcgtccc ggccgagtgc ttcgacctgc tgggccgcca ctgcgtggcc 120  
 tgcgggctcc tgcgcacgcc gcggccgaaa ccggccgggg ccagcagccc tgcgcccagg 180  
 acggcgctgc agccgcagga gtcggtgggc gcgggggccc gcgaggcggc gctgcccctg 240  
 cccgggctgc tcggtacc 258

<210> 76  
 <211> 235  
 <212> PRT  
 <213> Homo sapiens

<400> 76

Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser  
1 5 10 15

Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys  
20 25 30

Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr  
35 40 45

Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu  
50 55 60

Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser  
65 70 75 80

Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys  
85 90 95

Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys  
100 105 110

Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala  
115 120 125

Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro  
130 135 140

Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His  
145 150 155 160

Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala  
165 170 175

Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val  
180 185 190

His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr  
195 200 205

Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly  
210 215 220

Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp  
225 230 235

<210> 77  
<211> 185

<212> PRT  
<213> Homo sapiens

<400> 77

Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser  
1 5 10 15

Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys  
20 25 30

Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr  
35 40 45

Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu  
50 55 60

Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser  
65 70 75 80

Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys  
85 90 95

Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys  
100 105 110

Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala  
115 120 125

Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro  
130 135 140

Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His  
145 150 155 160

Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala  
165 170 175

Val Cys Thr Ser Thr Ser Pro Thr Arg  
180 185

<210> 78  
<211> 163  
<212> PRT  
<213> Homo sapiens

<400> 78

Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser  
1 5 10 15

Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys  
20 25 30

Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr  
35 40 45

Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu  
50 55 60

Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser  
65 70 75 80

Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys  
85 90 95

Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys  
100 105 110

Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala  
115 120 125

Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro  
130 135 140

Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His  
145 150 155 160

Gln Ile Cys

<210> 79  
<211> 142  
<212> PRT  
<213> Homo sapiens

<400> 79

Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser  
1 5 10 15

Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys  
20 25 30

Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr  
35 40 45

Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu  
50 55 60

Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser  
20



65

70

75

80

Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys  
                             85                            90                            95

Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys  
                             100                            105                            110

Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala  
                             115                            120                            125

Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys  
                             130                            135                            140

<210> 80  
 <211> 157  
 <212> PRT  
 <213> Homo sapiens

<400> 80

Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val  
   1                            5                            10                            15

Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg  
                             20                            25                            30

Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu  
                             35                            40                            45

Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe  
   50                            55                            60

Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile  
   65                            70                            75                            80

Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala  
                             85                            90                            95

Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys  
                             100                            105                            110

Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys  
                             115                            120                            125

Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe  
                             130                            135                            140

Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu  
   145                            150                            155

<210> 81  
<211> 51  
<212> PRT  
<213> Homo sapiens

<400> 81

Glu Pro Pro Thr Gln Lys Pro Lys Lys Leu Val Asn Ala Lys Lys Asp  
1 5 10 15

Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser Arg Leu Asp Thr  
20 25 30

Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln Ala Leu Gln Thr  
35 40 45

Val Cys Leu  
50

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☒ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**